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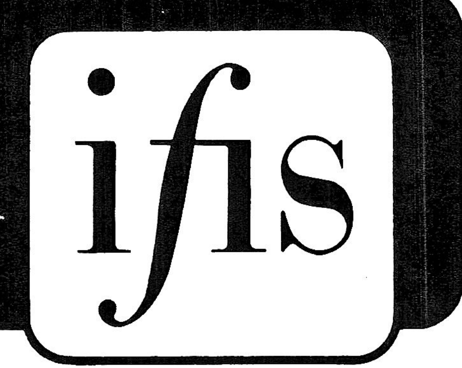

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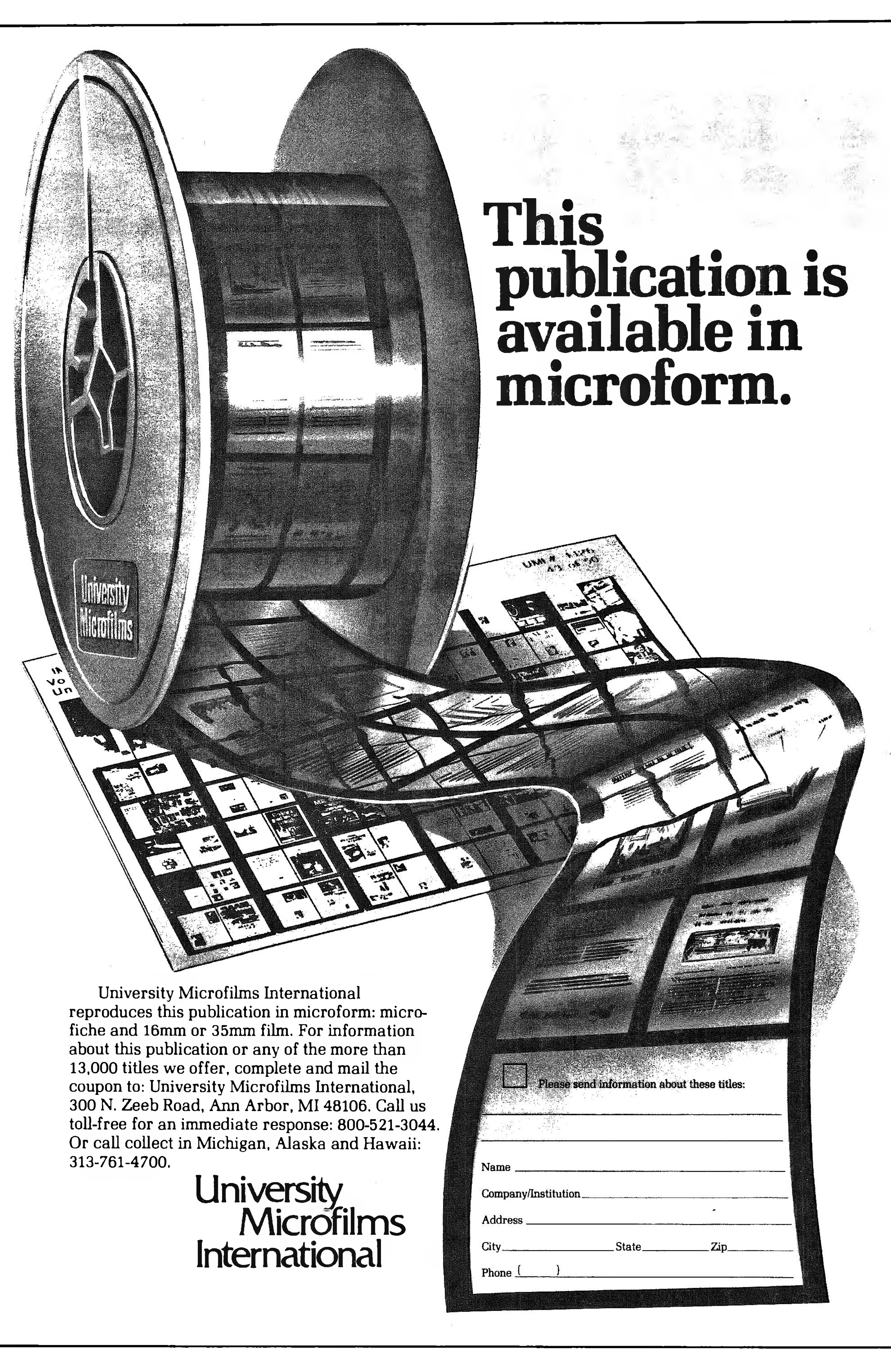

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Steady Shear Rheology and Fluid Mechanics of Four Semi-Solid Foods

MURAT DERVISOGLU and JOZEF L. KOKINI

ABSTRACT

The steady shear rheology of four semi-solid food materials (ketchup, mustard, apple sauce and tomato paste) was studied by generating and comparing flow data using cone and plate, parallel plate and capillary measurements over the shear rate range of 0.05–1600 sec^{-1}. Superposition of flow data was demonstrated and correlations obtained; using the power law, Bingham Plastic, Herschel-Bulkley and Casson models were compared. In the low shear rate range ($\dot{\gamma}$ = 0.05 – 1.0 sec^{-1}), the Bingham Plastic model and in the high shear rate range ($\dot{\gamma}$ = 1.0 – 1600 sec^{-1}), the Herschel-Bulkley model were shown to be most successful. Comparison of average velocity data with mathematical predictions using the power law, Herschel-Bulkley and Casson models showed that the equation using the Herschel-Bulkley model was best and should be used in the design of flow processes for the foods studied.

INTRODUCTION

RELIABLE and accurate steady shear rheological data are necessary for the design of continuous flow processes where flow rates or pressure drops in pipes and other flow systems must be predicted. Pressure drops in turn are used to size pumps and other fluid moving machinery; flow rates and velocity profiles are prerequisite to estimate heating rates during aseptic processing, concentration (Rao, 1977a, b; Sheath, 1976; Holdsworth, 1971). Measurement of rheological properties becomes particularly tedious in the case of semi-solid foods due to limitations arising from time dependency (Dzuy and Boger, 1983; Kraynik and Showalter, 1981; Figoni and Shoemaker, 1981; Kokini and Dickie, 1981; Prentice and Huber, 1983). They are further complicated by wall effects such as slip and wall pinch (Chong et al., 1971; Goto and Kuno, 1982) and secondary flows arising at relatively small shear rates in narrow gap geometries.

At present the flow characteristics of semi-solid foods are not fully understood. Even though there is a lot of data in the literature (Rao et al., 1981; Barbosa-Canovas and Peleg, 1983; Saravacos, 1968; Mizrahi and Berk, 1972; Charm, 1960; Rozema and Baverloo, 1974; Dickie and Kokini, 1983; Kokini and Dickie, 1981), available data usually result from rheological measurements using a particular technique in a limited shear rate range (Table 1). Furthermore, data obtained using different measurement techniques have never been compared. This is particularly important if data obtained using all kinds of rheometers are to be used in the design of flow processes.

The rheological models obtained using these data can be useful in design of food engineering processes if used in conjunction with mass, momentum and energy balances for a particular processing operation and are able to predict actual process parameters such as pressure drops, friction factors and velocity distributions. Without the availability of such design equations, selection and sizing of equipment are largely a trial and error procedure.

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The objectives of this research were: (1) to generate and compare rheological data for a wide shear rate range of four semi-solid foods: ketchup, mustard, apple sauce and tomato paste using capillary, cone and plate and parallel plate measurements, (2) to test the usefulness of three commonly employed rheological models — power law, Herschel-Bulkley and Casson, (3) to test predictions of each rheological model solved in conjunction with momentum and mass balances to estimate average velocities in pipe flow.

MATERIALS & METHODS

THE FOOD MATERIALS selected consisted of hot break tomato paste (supplied by the Campbell Soup Company), Hunt's Ketchup, Guilden's mustard and White House apple sauce. Rheological measurements of each food material were carried out at steady state in a capillary rheometer and the cone and plate and parallel plate geometries of the Rheometrics Fluids Rheometer. The capillary rheometer was custom built and different flow rates of the sample were attained by varying the air pressure in a vessel (Fig. 1). Flow rates were determined by measuring mass flow rate in a time interval of 20 sec. Densities necessary to convert mass flow rates to volumetric flow rates were measured by using a flow displacement technique using a buret. Pressure drop values corresponding to different sample flow rates were determined by taking the difference between the values of pressure measured by two transducers placed on a capillary 64 diameters apart. The pressure transducers used were Kulite XTM 190-250 model miniature diaphragm type and were flush mounted to avoid hole pressure error. An entrance length of 90 diameters was allowed to minimize entrance effects. This length was reached after trial and error experiments which gave entrance length independent rheological properties. The temperature of the food was kept constant at 23°C. Pressure drop values were measured for food materials corresponding to shear rates ranging from 0.3–1600 sec^{-1}. The presence of laminar flow in this geometry was verified by calculating Reynolds numbers and assuring that they were lower than 2100.

Steady state measurements with the cone and plate geometry were conducted using a 2.5 cm diameter cone with an angle of 0.0196 radiams. Torque values were measured with a 100 g-cm transducer. Cone and plate measurements were best suited in the shear rate range 0.05–10 sec^{-1} because of secondary flows. For these measurements the size of the solid particles was a limiting factor. It was not possible to obtain rheological data for apple sauce and tomato paste because particle to plate contact controlled the resistance to deformation. This was consistent with experimental difficulties encountered by Bongenaar et al. (1973).

The parallel plate geometry was used with a gap of 500μ for ketchup and mustard. Gap size dependency was investigated at gap sizes of 500, 1000, 1500, 2000 in the cases of tomato paste and apple sauce. Gap sizes of 1000 and 1500μ were used for tomato paste and apple sauce respectively. Shear stresses and shear rates were calculated using well known equations reported in the excellent treatise by Bird et al. (1977).

Average velocity versus pressure drop data were obtained in an independent experiment using a 1.25 cm i.d. galvanized steel pipe. The procedure was similar to that used with capillary measurements. Microscopic examination was conducted using a Bausch & Lomb microscope at ×400 magnification.

RESULTS & DISCUSSION

Effect of gap size in parallel plate measurements

Gap size in the parallel plate geometry was found to significantly affect rheological measurements. This was thought to

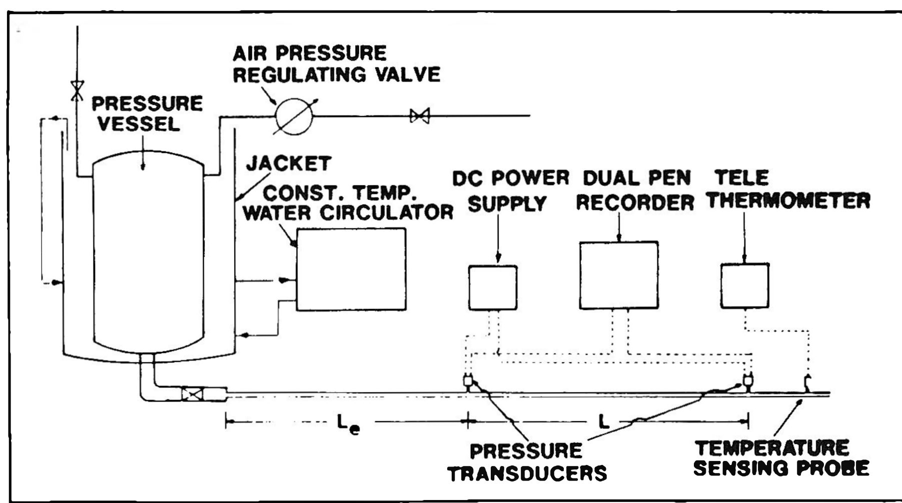

Fig. 1 — Schematic diagram of the capillary set-up.

Table 1 — Rheological data for ketchup, mustard, apple sauce, and tomato paste

Material	Temp (°C)	Technique	Shear rate range (sec^{-1})	Model	Parameters m(Pa – S^n	n	τ (P$_a$)	Reference
Ketchup	30.5	Cone and Plate	0.10100	Power Law	29.1	0.136	—	Dickie and
								Kokini (1983)
	25.0	Concentric Cyl.						Higgs and
		Smooth Cup	—	Herschel-Bulkley	18.7	0.277	32.0	Norrington (1971)
		Serrated Cup	—	Herschel-Bulkley	21.0	0.262	41.0	
	25.0	Concentric Cyl.	1.0–300	Herschel-Bulkley	2.2	0.61	24.0 (Brand A)	Barbosa-Canovas
					2.0	0.60	15.0 (Brand B)	and Peleg (1983)
				Casson	$k_o = 4.9$;	$k_c = 0.29$	(Brand A)	
					$k_o = 4.0$;	$k_c = 0.27$	(Brand B)	
								Dickie and
Mustard	30.5	Cone and Plate	1.0–100	Power Law	35.1	0.196	—	Kokini (1983)
	25.0	Concentric Cyl.	40.0–1100	Power Law	19.3	0.310	—	Rozema and
		Capillary	30.0–1300	Power Law	13.5	0.390	—	Baverloo (1974)
	25.0	Concentric Cyl.						Higgs and
		Smooth Cup	—	Herschel-Bulkley	33.4	0.40	41	Norrington (1971)
		Serrated Cup	—	Herschel-Bulkley	34.2	0.41	55	
	25	Concentric Cyl.	1.0–300	Herschel-Bulkley	5.5	0.52	35 (Brand A)	Barbosa-Canovas
					3.4	0.56	20 (Brand B)	and Peleg (1983)
				Casson	$k_o = 6.4$;	$k_c = 0.34$	(Brand A)	
					$k_o = 4.8$;	$k_c - 0.32$	(Brand B)	
Apple Sauce	24	Merrill Viscometer	—	Power Law	0.5	0.645	— (Brand A)	Charm (1960)
		Capillary	—	Power Law	0.7	0.408	— (Brand B)	
								Saravacos
	30	Bookfield	—	Power Law	11.6	0.34	—	and Moyer (1967)
	27	Capillary	90–120	Power Law	12.7	0.28	—	Saravacos (1968)
								Barbosa-Canovas and
	25	Concentric Cyl.	1.0–300	Herschel-Bulkley	6.8	0.42	34 (Brand A)	Peleg (1983)
				Herschel-Bulkley	6.4	0.43	30 (Brand B)	
				Casson	$k_o = 6.5$;	$k_c = 0.24$	(Brand A)	
				Casson	$k_o = 6.1$;	$k_c = 0.25$	(Brand B)	
Tomato Paste	25	Concentric Cyl.	4.0–500	Power Law	100	0.259	— (Nova)	Rao et al. (1981)
					125	0.213	— (#475)	
					55	0.216	— (New Yorker)	
					50	0.233	— (#934 Hot Br.)	
					70	0.175	— (#934 Cold Br.)	
				Casson	$k_o = 7.7$;	$k_c = 0.369$	(#934 Cold Br.)	
					$k_o = 7.0$;	$k_c = 0.544$	(#934 Hot. Br.)	
								Harper and
	32.2	Concentric Cyl.	500–800	Power Law	12.9	0.405	—	El Sahrigi (1965)

be due to the size of the aggregates present in the four semisolid materials. Microscopic examination of these materials revealed that for apple sauce, particle sizes ranged between 150μ to 400μ while the average size was 240μ. Tomato paste consisted of a continuous matrix containing aggregates with sizes ranging from 30–120μ with an average size of 75μ. Ketchup and mustard contained aggregates and particles ranging in size from 5–15μ and to 2–5μ, respectively. The effect of gap size for apple sauce is shown in Fig. 2. At the smallest gap size of 500μ shear stress values are largest suggesting that particle to plate contact controls the resistance to flow. For gap sizes larger than 1000μ, shear stress measurements no longer depended on gap size. The effect of gap size in the case of tomato paste is shown in Fig. 3. With tomato paste the effect of particle to plate contact was also observed for gap sizes smaller than 400μ. At gap sizes larger than 500μ, measured shear stress values for tomato paste kept on increasing with increased gap size at constant shear rate. A gap size where shear stress values were constant was not attained in this case. This is thought to be due to the dependence of shear stress values on structure breakdown during loading. As the gap is increased, structure breakdown due to loading decreases since the sample does not need to be squeezed as much. This is in contrast with apple sauce where the opposite behavior was

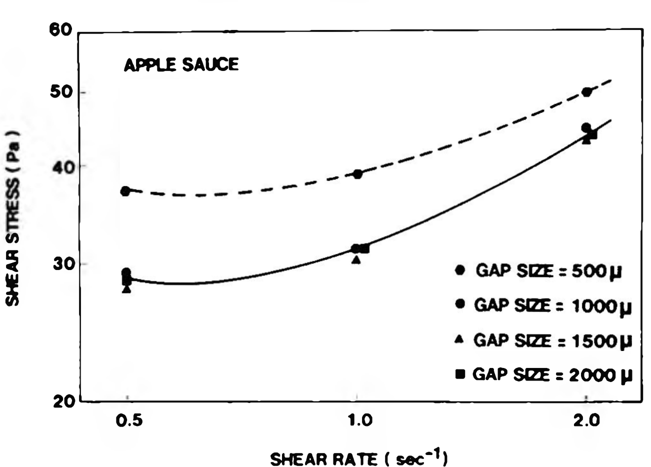

Fig. 2 — Gap size dependence of parallel measurements for apple sauce.

GAP SIZE DEPENDENCE OF PARALLEL PLATE DATA

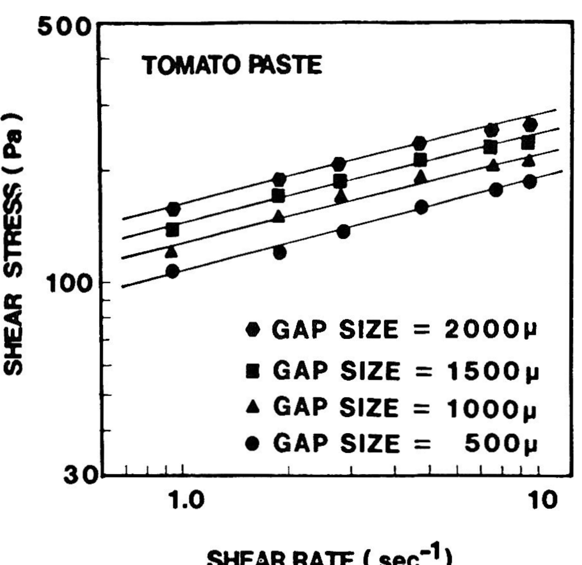

Fig. 3 — Gap size dependence of parallel plate measurements for tomato paste.

Fig. 4 — Shear stress vs shear rate data for ketchup and mustard.

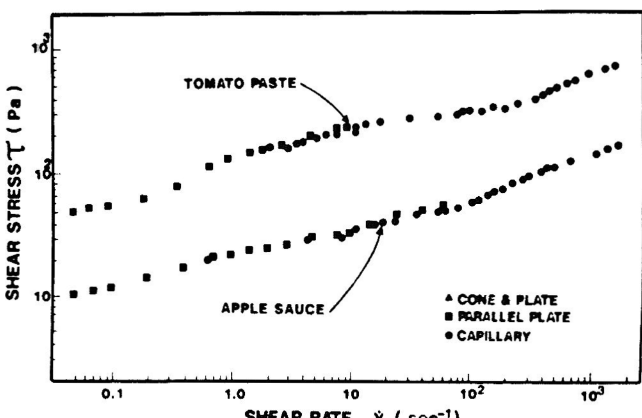

Fig. 5 — Shear stress vs shear rate data for apple sauce and tomato paste.

observed. Gap size dependency of parallel plate data was not observed with mustard and ketchup. This was thought to be due to particle sizes of 2–5μ which were much smaller than the gap size of 500μ.

Rheological Data

Shear stress vs shear rate data obtained for mustard and ketchup are shown in Fig. 4; those for tomato paste and apple sauce are shown in Fig. 5. It can be seen from both figures that superposition of cone and plate and parallel plate measurements was observed for all four materials. Capillary flow measurements on the other hand gave somewhat lower values especially in the high shear rate region. This may be due to the fact that dispersed solid particles tend to migrate away from the wall region which results in slip (Chong et al., 1971; Dzuy and Boger, 1983; Karnis et al., 1966; Serge and Silberberg, 1962). This aspect of food suspensions will be investigated further. However, measurements using capillary simulate the conditions that one would observe in pipe flow. If rheological measurements using narrow gap rheometers and capillary viscometers superimpose, then one could use the rheometer of choice for the particular application of interest. For example, when data are necessary at small shear rates, the cone and plate or parallel plate geometry could be used. This would be particularly useful to understand structure rheology relationships. On the other hand, if flow data at high shear rate are needed, then a capillary rheometer can be used.

Flow curves obtained for all four materials tend to a limiting stress value at small shear rates, demonstrating the presence of an apparent yield stress. When yield stress is subtracted from τ and $(\tau-\tau_o)$ plotted vs shear rate on logarithmic coordinates, the flow curves in Fig. 6 and 7 result. All of the flow curves portrayed a gradual transition from a less shear thinning behavior to a more shear thinning behavior with increased shear rate. This change in slope indicated that there was a gradual breakdown of aggregates generating a highly shear thinning structure at high shear rates compared to that in the lower shear rate region. This is consistent with observations on tomato juice obtained by DeKee et al. (1983), and those speculated by Charm (1960). At lower shear rates the time of shear was comparable to the time necessary to reform the aggregates and the forces exerted were small compared to the overall force necessary to achieve extensive breakdown. Consequently, the effective rate of breakdown was smaller than that observed in the larger shear rates. As a result of this gradual transition, two clearly different regions became evident. For all of the materials the less shear thinning region was observed for shear rates approximately less than 1.0 sec^{-1}. Even though the data have been plotted as $\tau-\tau_o$ vs $\dot{\gamma}$ on logarithmic coordinates, data in this region were correlated with power law, Casson and

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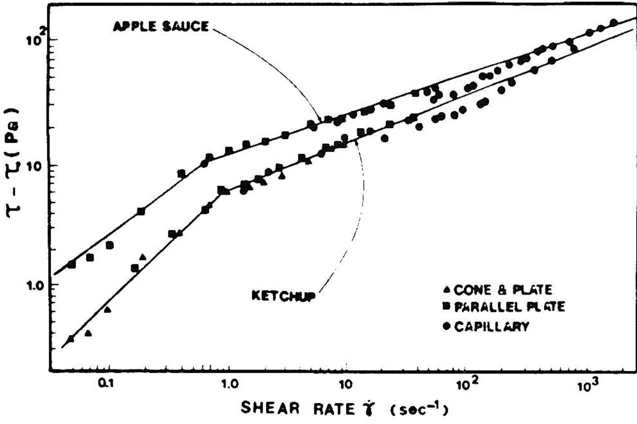

Fig. 6 — τ-τ_o vs shear rate data for ketchup and apple sauce.

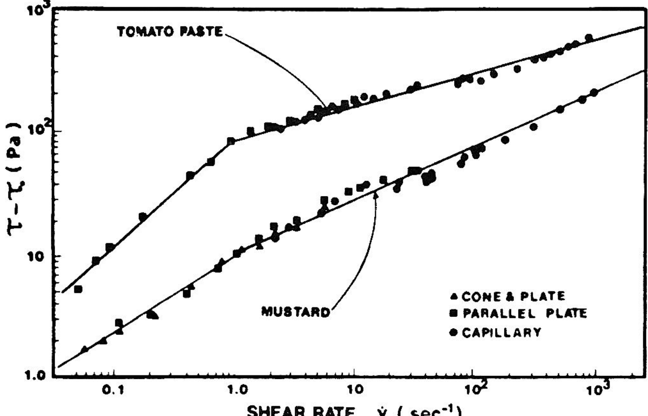

Fig. 7 — τ-τ_o vs shear rate data for mustard and tomato paste.

Table 2—Calculated model parameters in the shear rate range 0.005–1.0 sec^{-1}

Material	Model	Slope	Intercept	R^2	Apparent τ_o (Pa)
Mustard	Power Law	0.1095	222.2	0.889	—
	Casson	3.034	12.13	0.970	14.7
	Bingham Pl	70.270	165.2	0.995	16.5
Ketchup	Power Law	0.192	141.2	0.943	—
	Casson	4.10	8.09	0.993	6.5
	Bingham Pl	68.96	83.5	0.995	8.4
Apple sauce	Power Law	0.256	220.3	0.966	—
	Casson	6.92	8.7	0.994	7.6
	Bingham Pl	163.3	100.8	0.994	10.1
Tomato paste	Power Law	0.296	1137.4	0.966	—
	Casson	16.93	18.2	0.995	33.2
	Bingham Pl.	824.3	468.5	0.997	46.9

Bingham Plastic models (Table 2). In Table 2 it can be seen that for all four food materials studied the power law model is unsuccessful as demonstrated by low R^2 values. The Casson model was significantly less successful than the Bingham Plastic model for mustard as demonstrated by an R^2 value of 0.970 for Casson vs 0.995 for Bingham Plastic. For ketchup, apple sauce, and tomato paste on the other hand, the success of both Casson and Bingham Plastic models was similar as demonstrated by R^2 values which were very close. This was expected since both the Casson and Bingham Plastic models were two parameter models involving an apparent yield stress.

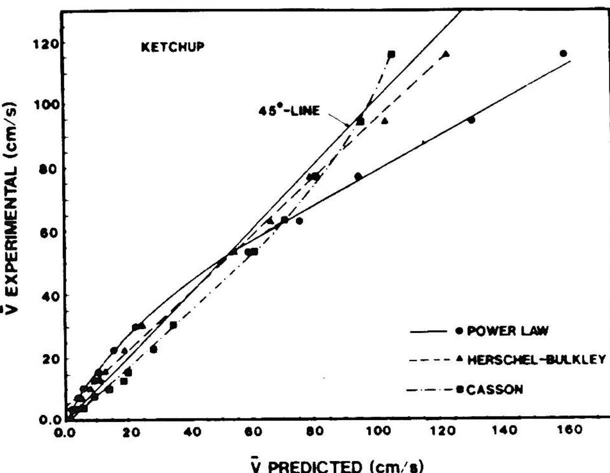

Fig. 8 — Experimental vs predicted average velocities for ketchup.

The region of shear rates of particular significance to processing is 1.0 to 1000 sec^{-1}. In Table 3 the success of the power law, Herschel-Bulkley and Casson models was tested. In this case for ketchup the Herschel-Bulkley model was slightly more successful than the Casson model and significantly more successful than the power law model. For mustard the Herschel-Bulkley model was again the most successful while the power law was second best and Casson third best. Similar results were obtained for apple sauce. For tomato paste the Herschel-Bulkley model was best but the Casson and power law models were equally satisfactory. The magnitude of the yield stresses in this study were comparable with those obtained earlier (Barbosa-Canovas and Peleg, 1983; Rao et al. 1981) but in most cases somewhat lower. This was due to the fact that in this work it was possible to measure shear stresses at shear rates as low as 0.05 sec^{-1}. This shear rate was approximately 20 times lower than the lowest shear rate attained by previous investigators. Furthermore, differences in brandnames and batches were undoubtedly quite significant. In all cases yield stresses obtained using the Casson model were much higher than those obtained experimentally as considered previously (Rao et al., 1981). It must be noted, however, that absolute values for yield stresses can only be attained through constant stress experiments, since measurement of yield stress using any stress obtained at even the lowest possible shear rate is beyond the true yield stress since flow has already occurred resulting in structure breakdown. This aspect of semi-solid foods is being investigated further (Fischbach and Kokini, 1984).

The parameters m and n were somewhat different in this work than those obtained earlier. The variation in these parameters was quite substantial in the literature and depended on the shear rate selected as well as brandnames. It was not possible to reconcile all these data. However, since in this work three different geometries have been used to generate rheological data for the brandnames selected, it can be assumed that the data was quite satisfactory if not superior to previous efforts.

Fluid mechanics

The results of fluid mechanics predictions of average velocity for steady laminar pipe flow obtained by using the power law, Herschel-Bulkley and Casson models provide the simplest test of usefulness of the models. Once a flow model is demonstrated to be superior to the alternatives in the circular cylindrical geometry, then appropriate equations for other geometries can be readily solved. Equations predicting average velocities for all three models are:

Table 3—Calculated model parameters in the shear rate range 1.0–1600 sec⁻¹

Material	Power law m (Pa·s)	Power law n	Power law R^2	Herschel-Bulkley τ_o (Pa)	Herschel-Bulkley m (Pa-s)	Herschel-Bulkley n	Herschel-Bulkley R^2	Casson K_o (Pa)½	Casson K_c (Pa-s)½	Casson R^2
Ketchup	13.05	0.280	0.962	8.0	6.53	0.382	0.978	4.14	0.211	0.972
Mustard	22.7	0.266	0.970	15.5	9.52	0.401	0.976	5.37	0.278	0.948
Apple sauce	18.4	0.271	0.972	9.0	11.51	0.334	0.980	5.30	0.213	0.954
Tomato paste	130.3	0.216	0.976	43.0	93.88	0.256	0.983	12.95	0.409	0.977

Power Law Model (Bird et al., 1960; Skelland, 1967)

$$v(r) = \left(\frac{\Delta P}{2Lm}\right)^{1/n}\left(\frac{n}{n+1}\right)\left(R^{\frac{n+1}{n}} - r^{\frac{n+1}{n}}\right)$$

$$\bar{V} = \frac{n}{3n+1}\left(\frac{\Delta P}{2Lm}\right)^{1/n} R^{\frac{n+1}{n}}$$

Herschel-Bulkley Model (Bird et al., 1960; Skelland, 1967)

$$v(r) = \frac{2Ln}{m^{1/n}(n+1)\Delta P}\left[(\tau_w - \tau_o)^{\frac{n+1}{n}} - \left(\frac{\Delta Pr}{2L} - \tau_o\right)^{\frac{n+1}{n}}\right]$$

$$\bar{V} = \frac{(AR-B)^{\frac{n+1}{n}}}{R^2 A^3}\left[\frac{n}{3n+1}(AR-B)^2 + \frac{2Bn}{2n+1}(AR-B) + \frac{B^2 n}{n+1}\right] \qquad A = \Delta P/2Lm \quad B = \tau_o/m$$

Casson Model

$$v(r) = \frac{4\,K_o}{3\,K_c^2}\left(\frac{\Delta P}{2L}\right)^{1/2}(r^{3/2} - R^{3/2}) - \frac{\Delta P}{4\,L\,K_c^2}(r^2 - R^2) - \frac{K_o^2}{K_c^2}(r-R)$$

$$\bar{V} = \frac{\Delta P\,R^2}{8\,L\,K_c^2} - \frac{4\,K_o}{7\,K_c^2}\left(\frac{\Delta P}{2L}\right)^{1/2} 5\,R^{3/2} + \frac{R\,K_o^2}{3\,K_c^2}$$

For all the four materials average predicted velocities were plotted against the experimentally determined average velocities (Fig. 8–11). If the prediction was perfect, the points would fall on the 45°-line. In the case of ketchup, predictions based on the power law model greatly deviated from the 45°-line, whereas predictions with Herschel Bulkley and Casson models were closer to the experimentally determined average velocities (Fig. 8). Furthermore, it can be shown that the Casson model predicts a minimum in average velocities. The value of this average velocity can be estimated from:

$$\bar{V}_{min} = 0.0068\,R\,(K_o/K_c)^2$$

This predicted minimum is a function of the square of the ratio of the two material properties K_o and K_c. This is an unrealistic result since average velocity is not expected to decrease as ΔP increases. However, this limitation applies to small average velocities only. Minimum velocities were 1.54 cm/sec for ketchup, 2.47 cm/sec for apple sauce, 1.49 cm/sec for mustard and 4.3 cm/sec for tomato paste. The power law model even though somewhat successful at $\bar{V}$ less than 50 cm/sec deviated substantially from experimental data for $\bar{V}$ greater than 50 cm/sec. This was due to the absence of a yield stress term in this model.

In the case of mustard, predictions with the power law model are again far off especially at high velocities (Fig. 9). With the Herschel-Bulkley model the predictions are significantly closer to experimental average velocities compared with Casson model both at high and low average velocities.

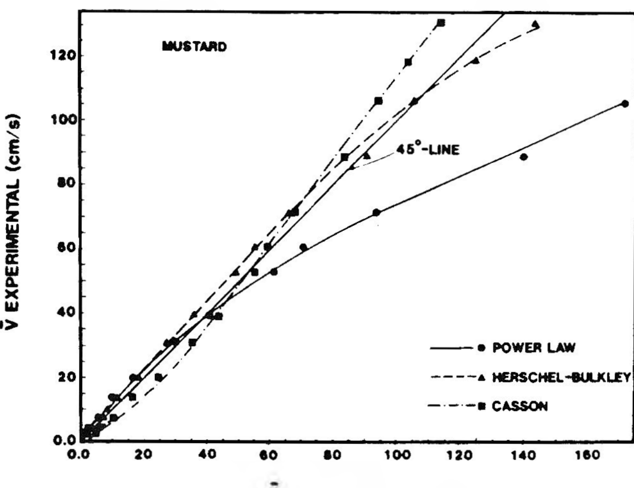

Fig. 9 — Experimental vs predicted average velocities for mustard.

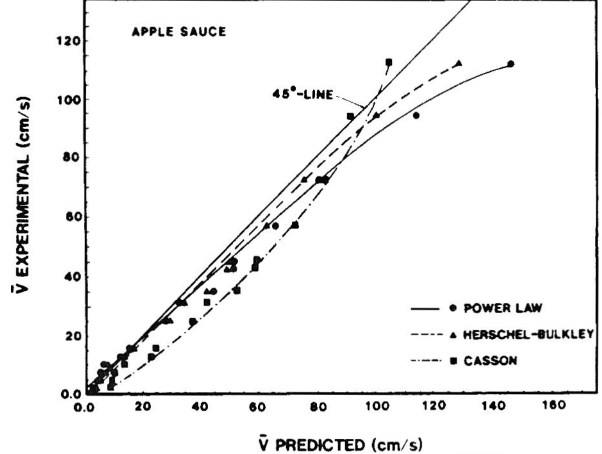

Fig. 10 — Experimental vs predicted average velocities for apple sauce.

In the case of apple sauce the power law provides close predictions at low velocities but the predictions are very far off at higher velocities (Fig. 10). Predictions based on Casson model were far off for almost the entire velocity range except for the two highest average velocities. Predictions with Herschel-Bulkley model were also close to the 45°-line within most of the velocity range; larger deviations were observed at the two highest velocities.

In the case of tomato paste average velocity predictions with all three models show considerable deviation from experimental data (Fig. 11). The Herschel-Bulkley and power law models were approximately equally deviant at all average velocities with the Herschel-Bulkley model coming closer to experimen-

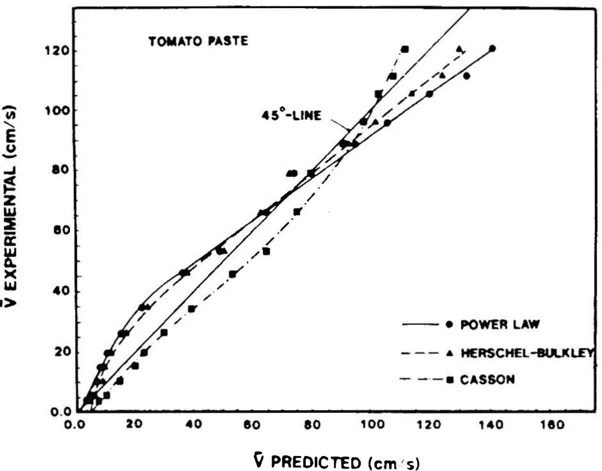

Fig. 11 — Experimental vs predicted average velocities for tomato paste.

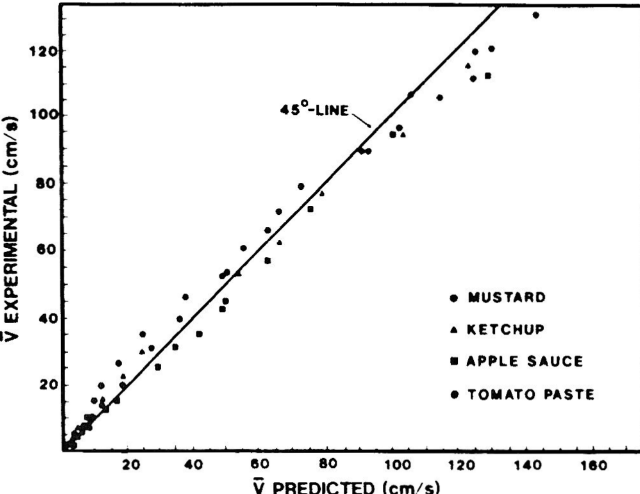

Fig. 12 — Experimental vs predicted average velocities using the Herschel-Bulkley Model for ketchup, mustard, apple sauce and tomato paste.

tally observed average velocities. At high velocities the Herschel-Bulkley model seemed to remain closer to experimental data. The Casson model on the other hand was somewhat more successful for $\bar{V}$ less than 50 cm/sec.

In order to compare the overall success of each individual model for all four materials linear regression parameters and coefficients of determination were calculated for each set of predicted vs experimental average velocity data. For ketchup the coefficient of determination was highest for the Herschel-Bulkley model and the slope and intercept were close to their ideal values (Table 4). The same was true of mustard and apple sauce even though the coefficient of determination using the power law model and the Herschel-Bulkley model was equal. However, the slope of the line of best fit and the intercept were much closer to their ideal values in the case of the Herschel-Bulkley model. For tomato paste the Casson model seemed to give the highest R^2 and the best slope.

To propose the best overall model for all four semi-solid food materials studied, predictions of each model for all four foods were combined as shown in Fig. 12. Then overall coefficients of determination as well as regression parameters were obtained as shown in Table 5. As shown in this table the Herschel-Bulkley model with an R^2 of 0.989 and slope and intercept values close to the ideal values was best suited for design purposes. Figure 12 shows the overall prediction for the Herschel-Bulkley model.

Table 4—Comparison of the validity of the Power Law, Herschel-Bulkley and Casson Models in predicting average velocities in laminar pipe flow

Material	Model	Intercept	Slope	R^2
Ketchup	Power law	3.56	0.73	0.985
	Hershel-B.	1.40	0.93	0.997
	Casson	−3.81	1.03	0.989
Mustard	Power Law	4.73	0.55	0.961
	Herschel-B.	1.46	0.96	0.994
	Casson	−3.67	1.14	0.994
Apple sauce	Power law	0.83	0.85	0.994
	Herschel-B.	−0.02	0.91	0.993
	Casson	−3.68	0.89	0.966
Tomato paste	Power law	4.03	0.89	0.973
	Herschel-B.	3.19	0.94	0.982
	Casson	−5.79	1.04	0.994

Table 5—Comparison of the overall success of rheological models in predicting average velocities for ketchup, mustard, apple sauce, and tomato sauce

	Rheological model		
Coefficient	Power Law	Herschel-Bulkley	Casson
Intercept	5.275	1.413	−4.650
Slope	0.668	0.943	1.039
R^2	0.921	0.989	0.978

CONCLUSION

THE USEFULNESS of the available techniques for rheological measurements was very much influenced by the structural properties of semi-solid foods. Rheological data obtained using different measurement techniques can be superimposed when these techniques are applied with the knowledge and consideration of relevant structural properties. The steady rheology of ketchup, mustard, apple sauce, and tomato paste can best be modeled as Bingham Plastic in the low shear rate range and as a Herschel-Bulkley material in the high shear rate range.

Accurate average velocity predictions necessitated the use of a rheological model which involved an apparent yield stress. The best fit of average velocity data was achieved using Herschel-Bulkley model.

NOMENCLATURE

K_o, K_c	Casson Model Parameters ($Pa^{0.5}$), ($(Pa\text{-}sec)^{0.5}$)
L	Length of the Tube (meters)
m	Consistency Index (Pa-s^n)
n	Flow Behavior Index
ΔP	Pressure Drop (Pa)
r	Direction of Velocity Gradient (meters)
R	Radius of the Tube (meters)
v	Local Velocity (meters/sec)
$\bar{V}$	Average Velocity (meters/sec)
Greek	
$\dot{\gamma}$	Shear Rate (sec^{-1})
τ	Shear Stress (Pa)
τ_o	Apparent Yield Stress (Pa)

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HPLC Method for Determination of Inositol Tri-, Tetra-, Penta-, and Hexaphosphates in Foods and Intestinal Contents

A.-S. SANDBERG and R. AHDERINNE

ABSTRACT

A HPLC method was developed for separation and quantitative determination of inositol tri-, tetra-, penta-, and hexaphosphates. The method included extraction of inositol phosphates with HCl, separation of the inositol phosphates from the crude extract by ion-exchange chromatography, and ion-pair C18 reverse phase HPLC analysis using formic acid/methanol and tetrabutylammonium hydroxide in the mobile phase. The inositol 3-6 phosphates of raw and extruded bran, soy flour, and intestinal contents were determined by HPLC and compared to phytate determinations by two iron precipitation methods. Inositol 3-5 phosphates were found in extruded products and intestinal contents. The HPLC method was rapid and gave reproducible values, which differed from those obtained by the precipitation methods in some samples.

INTRODUCTION

MOST METHODS for the determination of phytate are derived from the method of Heubner and Stadler (1914) and are based on the precipitation of ferric ion with phytate in dilute acid solution and analysis of phosphorus or iron in the precipitate (assuming a certain ratio of iron to phytate phosphorus). One of the disadvantages of the iron precipitation methods is that not only phytate (inositol hexaphosphate), but other phosphorus-containing compounds are precipitated. de Boland et al. (1975) found that all of the inositol phosphates from the di- to hexaphosphates form insoluble iron complexes. However, the mono-, di- and triphosphates are appreciably soluble and are not quantitatively precipitated. According to Møllgaard (1946), inositol mono- and diphosphates are not measured analytically by the iron precipitation methods.

In recent years, some HPLC methods for the determination of phytate have been developed (Tangendjaja et al., 1980; Camire and Clydesdale, 1982; Knuckles et al., 1982; Graf and Dintzis, 1982; Lee and Abendroth, 1983). Myo-inositol monophosphates have been determined by HPLC (Hallcher and Sherman, 1980) but not the inositol 2-5 phosphates. These compounds are usually separated by ion exchange chromatography (Bartlett, 1982) or paper chromatography (Sequi et al., 1966), both of which are time consuming methods. Due to the extreme polar nature of the inositol phosphates, quantitative gas chromatography is difficult (Leavitt and Sherman, 1982).

In mature plant seeds, the inositol phosphates occur mainly as hexaphosphate, but during food processes involving prolonged heat treatment, it is likely that other inositol phosphates are formed (de Boland et al., 1975). The decrease in phytate and the concurrent increase in intermediate inositol phosphates in yeast-raised bread has been observed by Nayini and Markakis (1983). During digestion of phytate in the stomach and small intestine it is most likely that lower inositol phosphates are formed and that food processing affects the digestibility (Sandberg et al., 1986). Precipitation methods for phytate determination in processed food and digesta are therefore inadequate as they lack the specificity to distinguish between the fully substituted (hexa)inositol phosphate and its partially dephosphorylated analogues.

The objective of this investigation was to develop a HPLC method for the quantitative determination of inositol tri-, tetra-, penta- and hexaphosphates in foods and intestinal contents. The method was applied to selected samples to study the occurrence of inositol phosphates in raw bran, an extrusion-cooked bran product, textured soy flour and intestinal contents.

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Material & Methods

Sample preparation

Samples of 0.5g freeze-dried, ground food or intestinal contents were extracted under vigorous mechanical agitation with 20 mL 0.5M HCl for 2 hr at 20°C (Graf and Dintzis, 1982). The extract was centrifuged and the supernatant decanted, frozen overnight and filtered through MF-Millipore (0.22 μm) under pressure. The inositol phosphates were separated from the filtrate and concentrated by a modification of the ion-exchange procedure of Graf and Dintzis (1982) as follows: plastic columns with glass filter and containing 0.65 mL resin (AG 1-X8, 200 - 400 mesh) were used. The filtrate was evaporated to dryness at reduced pressure and a water bath temperature not exceeding 40°C, dissolved in 20 mL 0.025M HCl and passed through the column (0.4 mL/min.) followed by 10 and 5 mL of 0.025M HCl. Inositol phosphates were removed from the resin with ten 1 mL portions of 2M HCl. The eluent was evaporated to dryness and diluted with 1 mL of water.

Standards

A stock phytate solution was made by dissolving 2.30g sodium phytate (BHD Chemical Ltd, England) in 100 mL water. The sodium phytate used consisted of 870 μmol phytate/g solid as determined by a phosphorus analysis of the wet-ashed solid. The linearity of phytate concentration versus peak area was investigated by 20 μL injections of solutions containing 2, 5, 8, 10, 16, 20 μmol/mL of phytate. Inositol triphosphate (Sigma Chem. Co, St. Louis, Mo), inositol pentaphosphate, and inositol hexaphosphate (Calbiochem-Behring, Co, San Diego, CA) were dissolved in water and used as reference compounds. Inositol tetraphosphate was not commercially available. A mixture of inositol phosphates was prepared by hydrolyzing sodium phytate. Hydrolysis was accomplished by refluxing 1g sodium phytate in 100 mL 0.5M HCl. Samples were taken out at different times (1–16 hr) to study the degradation of inositol hexaphosphate to lower inositol phosphates. Five milliliters of each sample were evaporated to dryness and dissolved in 10 mL water before injection. Sodium phytate was used as external standard.

Mobile phase

The mobile phase consisted of 0.05M formic acid: methanol (Fision HPLC grade) 49:51 and 1.5 mL/100 mL of TBA-OH (Tetrabutylammonium hydroxide) (Fluka, 40% in water) were added. The pH was adjusted to 4.3 by addition of 9M sulfuric acid. The mobile phase was filtered through a MF-Millipore filter (0.45 μm) under vacuum and degassed by sonication for 15 min.

HPLC procedure

A reverse phase C-18 column (Waters Radialpak) 5 micron particle size was equilibrated with the mobile phase for 1 hr. Analysis was conducted with an HPLC pump (Waters Model 510) equipped with a Z-module. Inositol phosphates were detected by refractive index (Waters

Differential Refractometer, Model R 401). The optimal flow rate was 3 mL/min. Retention times and peak areas were measured by a SP 4270 Integrator (Spectra Physics, Santa Clara, CA). Injections were made with a Rheodine 20 μL loop.

Inositol and phosphorus analyses

To identify inositol phosphates separated by HPLC the fractions of hydrolyzates and sodium phytate standard were collected and analyzed for phosphorus and inositol. The fractions were evaporated to dryness, diluted with 1 mL 6M HCl and hydrolyzed in screw capped tubes for 3 hr at 160°C, evaporated to dryness and diluted with 12 mL 0.27M H_2SO_4. An aliquot of 10 mL was analyzed for inositol by GLC of the inositol acetate according to the method of Theander and Åman (1979). The analysis was performed in a Perkin Elmer 990 gas chromatograph, with flame ionization detector, fitted with OV-275 capillary columns at a constant oven temperature of 200°C and a helium gas flow of 0.66 m/sec. The peak areas were measured with a SP 4100 Integrator (Spectra Physics). β-D-allose and glucose were used as internal standards.

One and one half milliliters of the hydrolyzate were evaporated and used for phosphorus analysis after wet-ashing in 0.25 mL sulfuric acid and 0.050 mL H_2O_2 for 2 × 15 min at 295°C. The digests were diluted with water to 10 mL and phosphorus was determined according to a modification of Fiske and Subbarow (1925). The analyses were performed in acid-washed glassware.

Analysis of food and intestinal contents

A raw coarse wheat bran and a mixture of 30% wheat bran, 60% starch and 10% gluten extruded under mild conditions were obtained from the Swedish Food Institute (SIK) and analyzed for inositol phosphates. Intestinal contents from an ileostomy subject consuming 54g per day of the extruded product or the corresponding raw mixture were analyzed. More detailed information about the experimental conditions of the ileostomy subject has been given in a previous paper (Sandberg et al., 1986). Textured soy flour (Supro 50, Purina Company, St. Louis, MO) was also studied.

RESULTS & DISCUSSION

TO ACHIEVE separation between the inositol tri- to hexaphosphates, the pH of the mobile phase was set at 4.3. A lower pH resulted in inadequate resolution between the inositol triphosphates and the solvent and too great a separation between inositol penta- and inositol hexaphosphates, while a higher pH resulted in inadequate resolution between inositol penta- and hexaphosphates. Inositol mono- and diphosphates could not be separated using this system.

The inositol triphosphate, pentaphosphate and hexaphosphate of the hydrolyzates and biological samples were identified by the retention times of the reference compounds. For further identification, HPLC fractions of inositol phosphates from the hydrolyzates and sodium phytate standard were analyzed for inositol and phosphorus after hydrolysis with 6M HCl. The stability of free inositol when exposed to strong HCl allows conditions adequate for complete hydrolysis (McKibbin, 1959), which was confirmed by analysis of the phosphorus and inositol recovered from sodium phytate after hydrolysis for 3 hr at 160°C. Six replicate collections of fractions from the same hydrolyzate were made and the results given as mean ± S.D. The fraction of hydrolyzates identified by the retention time as inositol triphosphate was found to contain phosphorus and inositol in a molar ratio of 3.2 ± 0.56 and the fraction supposed to be inositol tetraphosphate contained inositol and phosphorus in a molar ratio of 4.5 ± 0.69. The fractions identified by retention times as inositol pentaphosphate and inositol hexaphosphate were found to contain phosphorus and inositol in molar ratios of 5.0 ± 0.68 and 6.1 ± 0.72, respectively. The HPLC fraction of sodium phytate standard contained phosphorus and inositol in a molar ratio of 5.9 ± 0.12.

Hydrolysis of sodium phytate led to characteristic decreases in the peaks for the higher inositol phosphates and increases in those for the lower inositol phosphates. The absence of these peaks after complete hydrolysis proved that they only represented inositol phosphates.

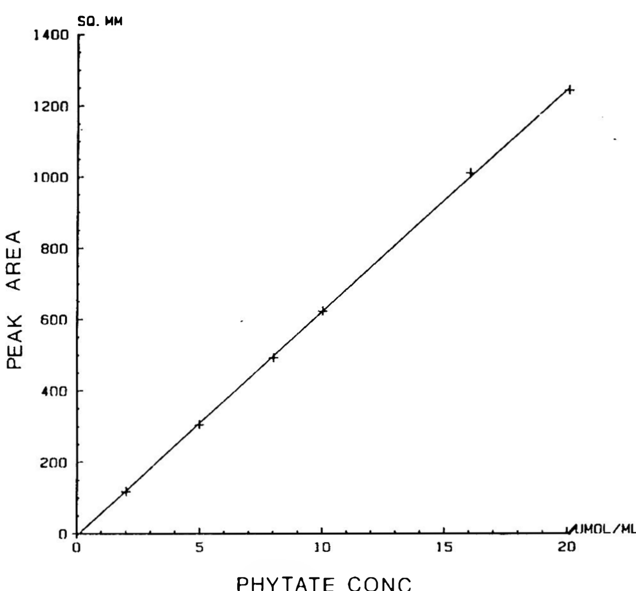

Fig. 1—Standard curve for phytate (6 phosphates per inositol residue). Each point represents the mean of three determinations. (r > 0.999). (20 μL injections of standard solutions).

The linearity of the phytate concentration versus peak area in the range between 0–20 μmol/mL was investigated. As demonstrated in Fig. 1, the area under the refractive index peak is linearly proportional to the phytate concentration over the entire range. The peak areas of inositol 3-5 phosphates of standards and hydrolysates were also found to be linear functions of concentration.

An internal standard similar to the inositol phosphates was difficult to find. The standards of inositol hexaphosphate and pentaphosphate were found to contain considerable amounts (10-15%) of penta- and tetraphosphates and were impossible to use as external standards. Therefore, the sodium phytate, which did not contain other inositol phosphates than the hexaphosphate, was used as external standard. The sodium phytate standard was also used for calculation of the tri-, tetra- and pentaphosphates. Correction factors for difference in detector response of inositol hexa, penta-, tetra- and triphosphates were calculated by comparison of peak areas of the HPLC fractions in relation to amount of inositol analysed by GLC in the collected inositol phosphate fractions. The correction factors for penta-, tetra-, and triphosphate were calculated to 1.1, 1.5, and 2.4, respectively. The correction factors were also calculated from peak areas in relation to inositol phosphates determined by phosphorus analysis and similar values were obtained.

When the bran samples were spiked with sodium phytate before extraction, the recovery of added phytate was approximately 100% in all samples. Recoveries of inositol tri, tetra-, penta-, and hexaphosphates of hydrolyzates and reference solutions from the AG 1-X8 column were investigated by comparison of peak areas before and after passing through the column. In all cases the recovery was found to be almost 100%. Graf and Dintzis (1982) used 0.1M HCl to place the inositol hexaphosphate on the AG 1-X8 column. However, the inositol triphosphate but not the tetra-, penta-, and hexaphosphate was partly eluted at concentrations of 0.05M HCl or above. With concentrations between 0.025M and 0.04M HCl, no decease in binding between the resin and the inositol triphosphate or the other inositol phosphates was found. In agreement with Graf and Dintzis (1982), the evaporation step did not influence the recovery.

HPLC analyses of raw wheat bran and extruded bran product are shown in Fig. 2, 3, and Table 1. The raw bran-gluten-

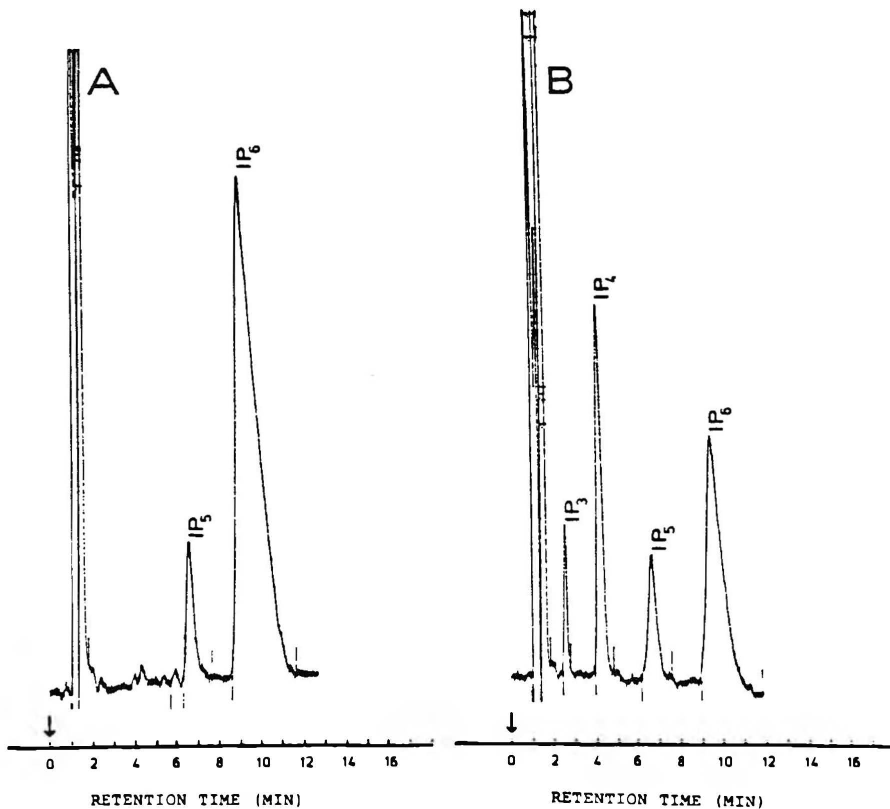

Fig. 2—Inositol phosphates in raw wheat bran (A) and after digestion in the stomach and small intestine of an ileostomy subject (B). IP_3 to IP_6 = inositol containing 3 to 6 phosphates per inositol residue. (20 μL injections of extracts.)

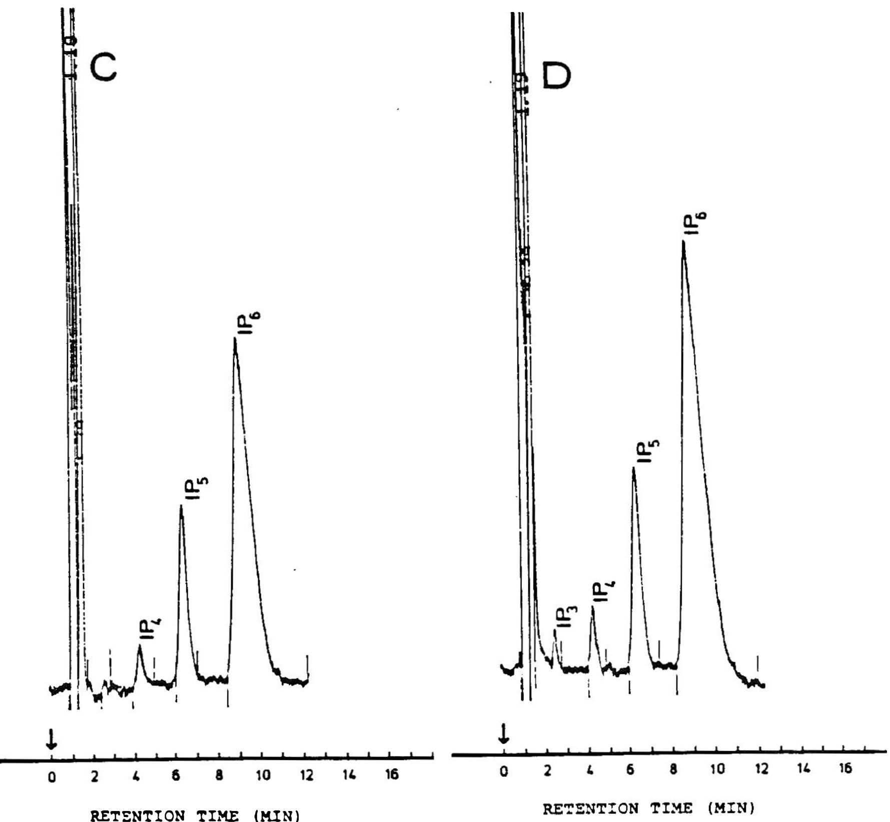

Fig. 3—Inositol phosphates in an extruded bran product (C) and after digestion in the stomach and small intestine of an ileostomy subject (D). IP_3 to IP_6 = inositol containing 3 to 6 phosphates per inositol residue. (20 μL injections of extracts.)

starch mixture was found to contain inositol penta- and hexaphosphate in proportion to the amount of raw bran included. After extrusion cooking the amount of inositol hexaphosphate decreased and the amounts of inositol tetra- and pentaphosphates increased. The peaks were identified by retention times and for wheat bran also by analysis of phosphorus and inositol in collected fractions from HPLC. The corresponding intestinal contents, after consumption of the raw bran - gluten - starch mixture, and the extruded product were also analysed for inositol phosphates (Fig. 2 and 3). The inositol hexaphosphate

Table 1—Inositol phosphates (μmol/g) and phytate content (μmol/g) of selected biological samples

						Phytate[c]	
Sample[a]	IP_3^b	IP_4	IP_5	IP_6	Sum of IP_3–IP_6	Ellis *et al.* (1977) Method	Davies & Reid (1979) Method
Raw wheat bean	–	–	5.7 ± 0.91	53.1 ± 1.71	58.8 ± 1.88	75.5 ± 0.46	58.6 ± 1.4
Extruded bran product	–	0.5 ± 0.18	3.1 ± 0.08	12.2 ± 0.26	15.8 ± 0.44	22.4 ± 0.45	18.3 ± 0.30
Textured soy flour	–	0.9 ± 0.29	4.4 ± 0.67	21.8 ± 1.25	21.8 ± 1.25	27.5 ± 0.69	29.7 ± 0.60
Intestinal contents from bran period	2.7 ± 0.19	3.8 ± 0.16	1.3 ± 0.21	6.3 ± 0.23	14.1 ± 0.63	14.3 ± 0.56	not determined
Intestinal contents from extruded bran period	0.7 ± 0.07	0.5 ± 0.06	3.3 ± 0.14	16.4 ± 0.43	20.9 ± 0.27	26.9 ± 1.17	not determined

[a] Five replicate extracts of wheat bran and four replicate extracts of each other sample were prepared and analyzed in duplicate with HPLC. (Mean values with their standard deviations expressed on a dry weight basis.)

[b] IP_3 to IP_6 = inositol containing 3 to 6 phosphates per inositol residue.

[c] Each value represents the average of two or three determinations. It was not possible to use the method according to Davies and Reid (1979) for analysis of intestinal contents.

from raw bran was largely degraded to lower inositol phosphates in the intestinal contents, while the inositol phosphates from the extruded bran product were unchanged.

The phytate of the samples was also analyzed by the iron precipitation methods of Ellis et al. (1977) (modified by Sandberg et al., 1982) and Davies and Reid (1979) (Table 1). Even if calculated as the sum of inositol tri- to hexaphosphates, the value of phytate analyzed by the HPLC method differed from that obtained by the iron precipitation methods. This difference could be explained by the fact that the latter cause coprecipitation with other phosphorus compounds but further studies are needed to determine the basis of the difference observed in some samples. The HPLC method for inositol phosphates gave reproducible values in the samples studied (Table 1).

CONCLUSIONS

THE HPLC METHOD described gave consistent and reproducible values for inositol tri- to hexaphosphates of food and biological samples. The reproducibility and rapidity compared to ion-exchange chromatography and paper chromatography make it a useful method for analysis.

The method will be used for further studies on the digestion of phytate in humans and the effect of food processing on the formation of lower inositol phosphates.

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Temperature Dependence of Water Sorption Isotherms of Some Foods

HÉCTOR A. IGLESIAS, JORGE CHIRIFE, and CONSTANTINO FERRO FONTÁN

ABSTRACT

The effect of temperature on sorption isotherms of some foods may be taken into account by considering only the effect of temperature on the monolayer moisture content. Thus, knowing the variation with temperature of the monolayer (data are available in the literature) it is possible to predict the temperature dependence along the isotherm. The physicochemical foundation of this behavior is discussed on the basis of the Hailwood-Horrobin model for water sorption. It is suggested that temperature-induced structural modification in the substrate plays an important role in the observed phenomena.

INTRODUCTION

A FUNDAMENTAL CHARACTERISTIC of foods which influences many aspects of the dehydration process and the storage stability of the dried product is its water sorption isotherm. Several mathematical models have been reported in the literature to describe the sorption behavior of foods, and these were reviewed by Chirife and Iglesias (1978), Van den Berg and Bruin (1981) and Boquet et al. (1978). However, only few models have been able to adequately describe the temperature dependency of the isotherm (Iglesias and Chirife, 1976; Chen and Clayton, 1971; Ferro Fontán et al., 1982). Probably, the main reason for the limited success of the mathematical prediction of the temperature dependency of the isotherms, is the existence of some irreversible changes which occur in food materials subjected to increased temperatures (Bandyopadhyay et al., 1980). Because of these irreversible changes the conventional thermodynamic analysis of water sorption becomes very difficult. Since the nature and magnitude of temperature-induced changes suffered by the food substrate depend on its composition, to take these modifications into account in a general model is a very difficult task.

It is the purpose of the present work to introduce a simple method to predict the effect of temperature on the water sorption isotherm of some foods, and to discuss its theoretical implications.

RESULTS & DISCUSSION

IGLESIAS AND CHIRIFE (1984) recently showed that the following equation was very useful to correlate the dependence of B.E.T. monolayer moisture contents with temperature

$$\ln X_m = \beta + \alpha T \quad (1)$$

where X_m is B.E.T. monolayer moisture content (g water/100g dry solid), T is temperature (°C), and β and α are constants.

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Eq. (1) described very well the decrease of X_m with increasing temperature for a large variety of foods (37 different items including starchy foods, proteins and protein foods, fruits, vegetables and spices) in the approximate temperature range 5 to 60°C. Iglesias and Chirife (1984) calculated the best values of β and α for each food studied.

The basis of the method presented here consists in the hypothesis that the effect of temperature on sorption isotherm may be computed considering only temperature changes on the monolayer moisture content. For this purpose, the sorption isotherms of several foods at various temperatures were examined. A "reduced" isotherm for various foods was calculated as follows. The reduced equilibrium moisture content, defined as $\theta = m/X_m$ (*m* is moisture content), was calculated at each temperature and plotted versus water activity. It was found that for some foods the isotherms at different temperatures were brought approximately to the same curve when the sorption data were plotted in this way. Figure 1 illustrates this behavior for corn at several temperatures. For each food, for which this approach was reasonably successful, an average "reduced" isotherm was calculated and the results are shown in Table 1. It is to be noted that this is far from being a general behavior in food items, since only some of a large number of food isotherms examined followed the above behavior, which allowed for a relatively accurate prediction of the effect of the temperature. This will be discussed latter in connection with a physical water sorption model. In this manner, the sorption isotherm at any temperature may be obtained from the data in Table 1 and the knowledge of the monolayer moisture content at the desired temperature; this last value is readily obtained

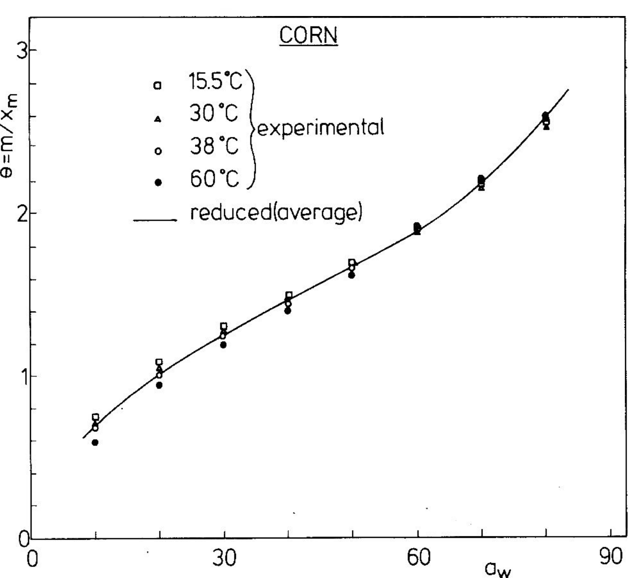

Fig. 1—"Reduced" adsorption isotherm for corn; experimental data were obtained from Saravacos and Stinchfield (1965).

Table 1—Reduced equilibrium sorption values

Water activity (a_w)	$\theta = m/X_m$								
	Raw beef[a] (ads.)	Turkey[b] (des.)	Corn[c] (des.)	Eggs,[d] dried (ads.)	Potato[e] (ads. and des.)	Sorghum[f] (ads.)	Wheat[g] starch (ads.)	Wheat[g] flour (ads.)	Fish[h] protein conc (ads.)
0.10	0.59	0.46	0.69	0.61	0.74	0.83	0.76	...	0.77
0.20	0.95	0.77	1.02	0.91	1.02	1.10	1.02	1.17	1.09
0.30	1.24	1.02	1.26	1.17	1.25	1.33	1.27	1.38	1.29
0.40	1.48	1.30	1.46	1.46	1.51	1.52	1.50	1.58	1.44
0.50	1.71	1.59	1.67	1.83	1.79	1.72	1.75	1.82	1.65
0.60	1.94	1.93	1.92	2.27	2.13	1.95	2.02	2.12	1.91
0.70	2.28	2.39	2.20	2.89	2.59	2.21	2.39	2.48	2.27
0.80	2.82	3.01	2.58	...	3.55	2.53	2.95	2.96	2.71

[a] Experimental data from Saravacos and Stinchfield (1965)
[b] Experimental data from King et al. (1968)
[c] Experimental data from Chen and Clayton (1971)
[d] Experimental data from Makower (1945)
[e] Experimental data from Gane (1950)
[f] Experimental data from Chen (1971)
[g] Experimental data from Bushuk and Winkler (1957)
[h] Experimental data from Rasekh et al. (1971)

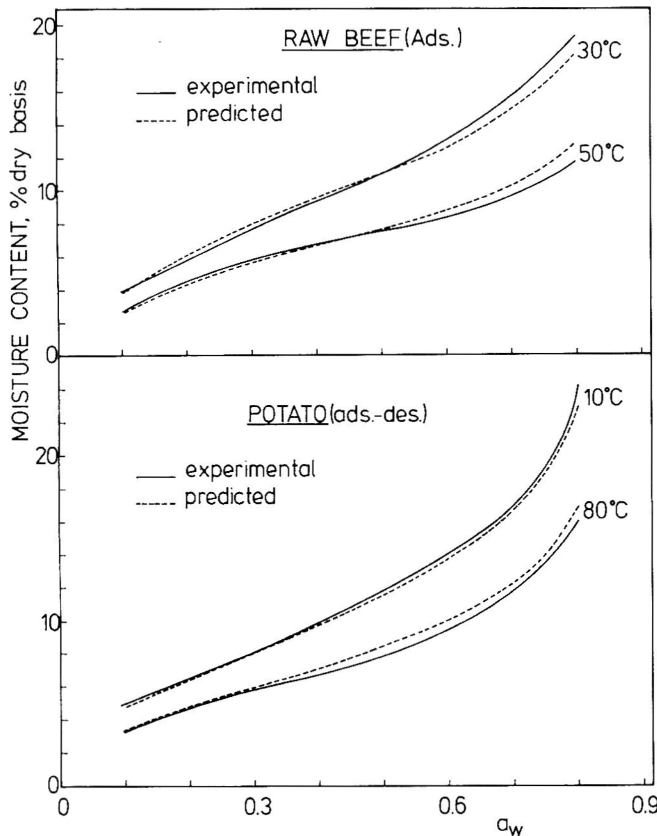

Fig. 2—Comparison of predicted and observed effect of temperature on adsorption isotherm of raw beef and potato; experimental data for raw beef are from Saravacos and Stinchfield (1965), and for potato from Gane (1950).

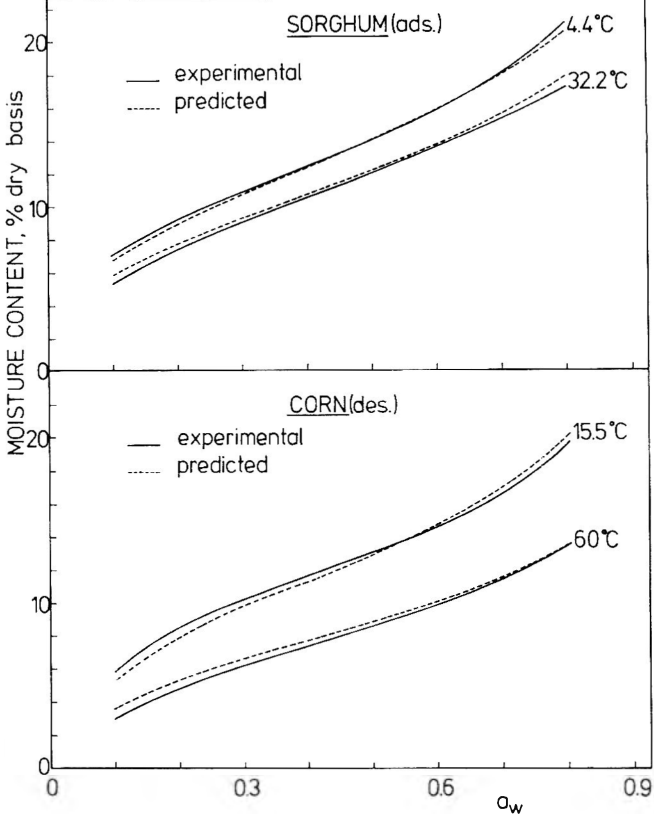

Fig. 3—Comparison of predicted and observed effect of temperature on adsorption isotherm of sorghum and desorption isotherm of corn; experimental data for sorghum and for corn were taken from Chen (1971), and Chen and Clayton (1971), respectively.

from Eq. (1) and published values of β and α (Iglesias and Chirife, 1984). Predicted and experimental isotherms at various temperatures are compared in Fig. 2, 3 and 4.

Theoretical analysis

The reason that the temperature dependence of some food isotherms may be approximately predicted using a "reduced" isotherm, may be explained as follows. The well known Hailwood and Horrobin (1946) isotherm equation may be written

$$\frac{M_s}{18}\frac{m}{a_w} = \frac{1}{\gamma - a_w} + \frac{1}{a_w + \gamma K} \quad (2)$$

where M_s is an average value of the molecular weight of a polar unit; a_w is water activity; and γ and K are interaction constants (Ferro Fontán et al., 1982). Eq. (2) may be "reduced" by setting

$$\bar{a}_w = \frac{1}{\gamma} a_w; \qquad \bar{m} = \frac{M_s}{18} m$$

and in B.E.T. notation

$$\frac{\bar{m}}{\bar{a}_w} = \frac{1}{1 - \bar{a}_w} + \frac{1}{\bar{a}_w + K} \quad (3)$$

It is noteworthy that $18/M_s$ is the monolayer value in the B.E.T. theory. It follows that Eq. (3) would be independent of temperature if the parameter K were not temperature-dependent. However, this is not the most common situation since K is related to temperature according to Ferro Fontán et al. (1982)

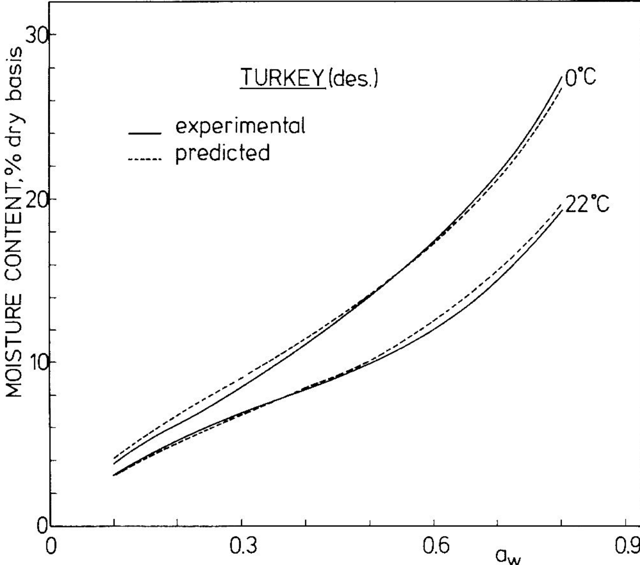

Fig. 4—Comparison of predicted and observed effect of temperature on desorption isotherm of turkey; experimental data were obtained from King et al. (1968).

$$K = \exp \quad \Delta G°/RT \tag{4}$$

and

$$\Delta G° = \Delta H° - T\Delta S° \tag{5}$$

Ferro Fontán et al. (1982) found that for various relatively simple food materials (i.e. proteins), K changed with temperature according to Eq. (4), while M_s (structure-related parameter) was only slightly temperature dependent. However, for various other foods, strong variations of M_s with temperature were observed, and this fact was attributed to structural modifications occurring in the substrate. Thus, depending on the food material — and also on the temperature interval considered — one may have different situations with respect to the effect of temperature on M_s and K. If the relative changes of M_s with temperature are more important than those experienced by the equilibrium constant, K, this latter may be assumed for fitting purposes to be almost invariant with temperature. In fact, Ferro Fontán et al. (1982) showed that a plot of Eq. (2) as (a_w/m) versus a_w was an inverted parabola whose zeroes were located near $a_w = 1$ ($a_w^{(o)} = \gamma$) and $a_w = 0$ ($a_w^{(o)} = -\gamma K$). Usually the position of the last one is temperature dependent, but for the cases considered in the present paper the dominant role is played by the temperature behavior of the vertex of the parabola, which is proportional to M_s. Thus, neglecting the variation of K with temperature produces only a slight disagreement at the lowest activities (Fig. 1), which can be easily compensated by the fitting algorithm. In other words, the reduced Eq. (3) would become more or less independent of temperature, which is the same as to say that the effect of temperature is accounted for by considering only the changes in the monolayer (i.e. M_s). This situation may occur in food substrates likely to suffer temperature-induced structural modifications and/or also having a low value of $|\Delta H°|$, and thus small relative variation of K with temperature, since

$$\frac{\Delta K}{K} = \frac{|\Delta H°|}{R\,T^2} \quad \Delta T$$

It is noteworthy that the so-called structural modifications may be identified with changes known to occur in heat-treated foods, such as changes in the crystallinity of polymers, cross-linking and denaturation of proteins, and nonenzymatic browning reactions, among other changes.

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Finite Element Modeling of Moisture Transfer During Storage of Mixed Multicomponent Dried Foods

Y. C. HONG, A. S. BAKSHI, and T. P. LABUZA

ABSTRACT

A finite element model is described that can be used for predicting moisture transfer in mixed food products during storage. Two of three components (raisins, roasted peanuts, banana chips, and roasted almonds) were packed in a well sealed cup. The rate of moisture transfer between the components was assumed to be limited by the component with the lowest effective diffusion coefficient divided by the square of half of smallest dimension (i.e., D_{eff}/L_o^2) value. The surface moisture content of the rate determining component was assumed to be in equilibrium with the other component(s) at all times. The mean error between the experimental and predicted moisture values with time ranged from 0.28% to 0.84% as a percentage of moisture content.

INTRODUCTION

THE MOISTURE CONTENT of a food strongly influences texture, storage stability vis-a-vis chemical reactions and susceptibility to microbial spoilage (Scott, 1957; Labuza, 1970, 1973, 1980; Troller and Christian, 1978). Since the concept of water activity (a_w) was introduced by Scott (1957) and Salwin and Slawson, (1959), it has been demonstrated that, in multicomponent foods, a transfer of moisture occurs from the component with a higher water activity to those at a lower water activity. At equilibrium all components will have the same water activity. The final moisture content of each component will influence the quality and shelf life of the mixed product.

Several mathematical models have been used to predict moisture transfer of foods. Crank (1975) derived differential equations for moisture diffusion for fixed geometries with constant moisture properties. These have been extensively used to predict drying time (Van Arsdel et al., 1973; Sherwood, 1936). Hsu (1981, 1983) and Singh et al. (1984) used the Finite Difference method to solve these differential equations for moisture gain/loss from spherical shapes. Another powerful method, the Finite Element method, has been used successfully by Zhang et al. (1984), Chhinnan and Bakshi (1984) and Lomauro and Bakshi (1985) to solve the differential equation for two-dimensional diffusion problems. The Finite Element method is a numerical procedure for solving differential equations using a minimization procedure. The advantages of this method are: the material properties in adjacent elements do not have to be the same; irregularly shaped boundaries can be approximated; the size of elements can be varied; and mixed boundary conditions can easily be handled (Segerlind, 1976).

All the studies mentioned above were for single component foods during drying, soaking, or storage. Little work has been done on the moisture transfer behavior of mixed multicomponent foods. For the more complex situation, one needs to determine how fast desorption or absorption of moisture is taking place and which component is rate limiting for moisture change. Thus, the objectives of this study were to develop a computer-aided model which could be used to predict moisture transfer in a multicomponent well mixed system during storage and to experimentally verify this model.

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Table 1—Initial moisture contents for each component in the mixtures

	Initial moisture content % (dry basis)		
System[a]	1st component	2nd component	3rd component
RP(25/25)	26.5	1.75	----
RP(25/30)	26.5	1.75	----
PA(30/20)	26.5	1.02	----
RA(25/25)	26.5	1.02	----
RA(20/30)	26.5	1.02	----
RB(25/15)	26.5	2.70	----
RB(20/20)	25.5	2.70	----
RB(30/10)	25.5	2.70	----
RPB(20/20/10)	26.5	1.75	2.70
RPA(20/15/15)	27.2	1.43	0.96
RPA(10/20/20)	27.2	1.43	0.96

[a] R = raisin; P = roasted peanut; A = roasted almond; B = banana chip (a/b/c): weight of each component in grams

MATERIALS & METHODS

ALL THE FOODS used in this study were purchased from a local food supplier. The materials chosen were raisins, roasted peanuts, banana chips, and slivered roasted almonds to represent the shapes of a finite cylinder (raisin, peanut), semi-infinite slab (banana chip), and finite slab (slivered almond). The raisins were prehumidified to a moisture content of about 27% (dry basis) at an a_w of 0.70. The effective diffusion coefficient (D_{eff}), characteristic length (L_o), and the moisture sorption isotherm parameters (GAB equation) were determined previously by Lomauro and Bakshi (1985) and Hong (1985). They used saturated salt solutions to generate a constant equilibrium relative humidity and used a nonlinear optimization technique to calculate the D_{eff} and GAB parameters. The moisture content of each component was determined by the AOAC (1984) vacuum oven method. Moreover, the procedure was duplicated for each package.

Different ratios of two or three food components were combined in 5 oz plastic cups covered with lids. Each package was then completely coated with white wax to prevent moisture transfer to the environment. The mixture information and initial moisture content of each package are shown in Table 1. These packages were then stored at a temperature of 25 ± 1°C. After a preset time period, a portion of these packages was unpacked, each component was separated, and the moisture content determined by the vacuum oven method. The samples were then discarded. There was no moisture transfer across the packaging materials as indicated by a negligible change in the total weight of each package. The moisture change of each component was predicted by using Finite Element Modeling. The details of the Finite Element Modeling approach are given in the next section.

The mean error between the experimental and predicted moisture of each component was calculated as a percentage of actual moisture content by the following formula:

$$\text{mean error} = \frac{\sum^{m}\sum^{n} |M_a - M_p|}{n \cdot m} \quad (1)$$

In Eq. (1), n is the total number of components, m is the total number of data points, M_a is the actual data point, and M_p is the predicted moisture content at that time. To evaluate the time needed to reach equilibrium, the moisture difference value (H) was calculated as:

$$H = \frac{|M_T - M_{T+13}|}{M_T} \cdot 100\% \quad (2)$$

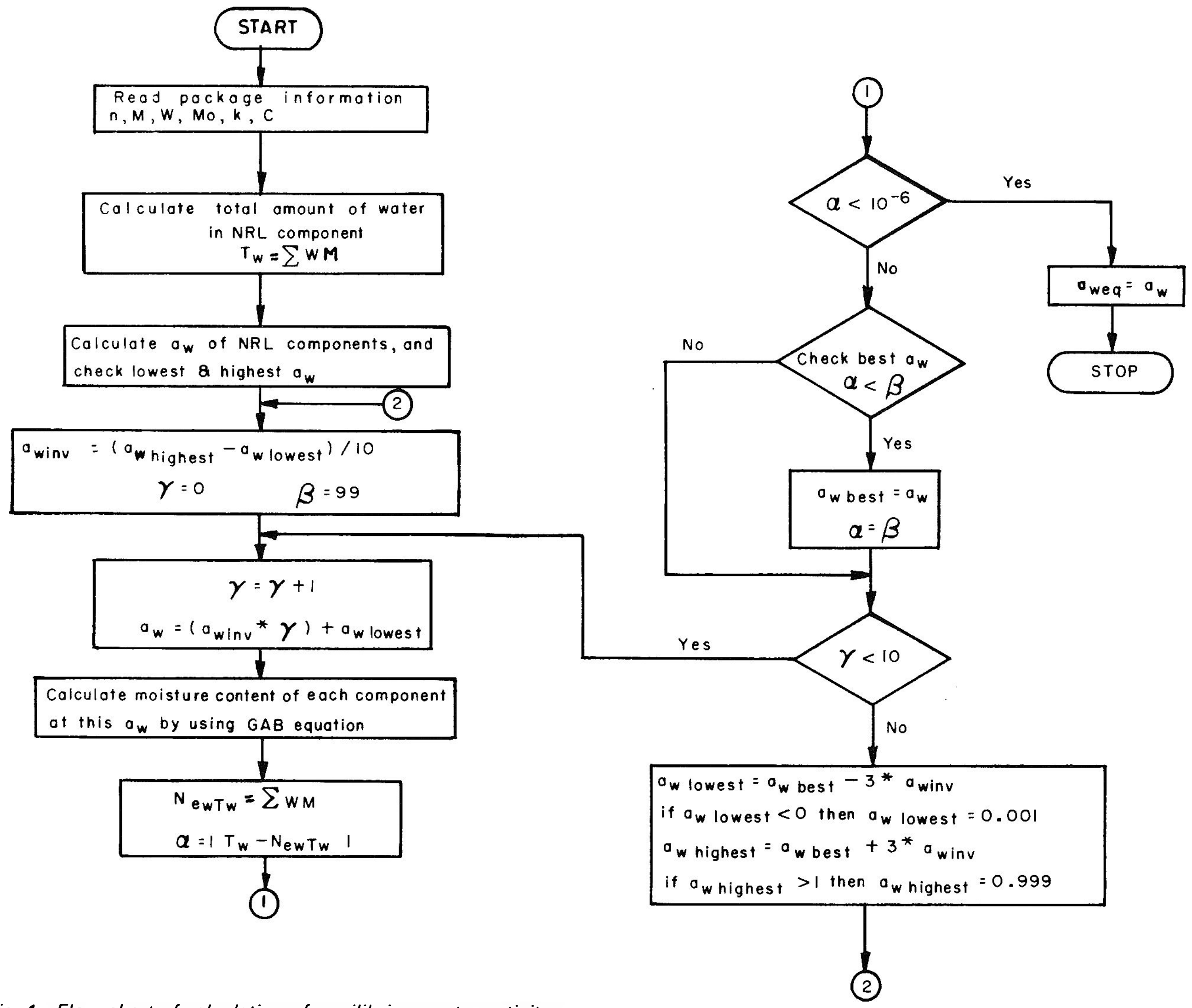

Fig. 1—Flow chart of calculation of equilibrium water activity.

Table 2—Properties for each component used in this study

Component	D_{eff} ($m^2/hr \cdot 10^8$)	L_o ($m \cdot 10^3$)	D_{eff}/L_o^2 ($hr^{-1} \cdot 10^5$)	GAB parameters		
				M_o	k	C
Raisin[a]	0.15	4.1	8.9	0.0764	1.0333	8.0650
Roasted peanut[b]	1.368	4.34	72.6	0.0130	1.1063	9.6474
Banana chip[b]	1.048	1.76	338.3	0.0196	1.0205	15.9114
Roasted almond[b] (slivered)	0.579	1.99	146.2	0.0142	1.1266	9.7665

[a] Data from Lomauro et al. (1985)
[b] Data from Hong (1985)

where M_T is the moisture content at time T days and M_{T+13} is the moisture content at time T + 13 days. Equilibrium was assumed to be reached when the H value of the rate-limiting component did not change by more than 2% over a 2-wk period, thus the time to reach equilibrium was considered as the time T (days). This was found to be an adequate measure of equilibrium by Lomauro et al. (1985) when compared to moisture gain after 6 months storage.

RESULTS & DISCUSSION

Finite element model for moisture transfer

For this model, the limiting factor in determining the rate of moisture transfer, was assumed to be the component with lowest effective diffusion coefficient for moisture divided by the square of half of the smallest dimension (i.e., D_{eff}/L_o^2). In each case, in this study, this represented the raisin. All the moisture transfer to this rate-limiting (RL) component was assumed to be absorbed from the other component(s). The accumulated moisture in the air space inside the package was assumed to be negligible as the amount of air is small. No moisture transfer across the container barrier was assumed.

The driving force for moisture transfer was assumed to be a liquid concentration gradient. The effective diffusion coefficient, dry matter density, and temperature of the component were assumed to be constant. The moisture transfer in a two-dimensional axisymmetric body is governed by (Crank, 1975):

$$r D \frac{\partial^2 M}{\partial r^2} + D(\frac{\partial M}{\partial r}) + \frac{\partial}{\partial z}(r D \frac{\partial M}{\partial z}) = r \frac{\partial M}{\partial t} \quad (3)$$

The initial condition for the moisture content for the RL component is:

$$M(r, z, 0) = M_i, \text{ at } t = 0 \quad (4)$$

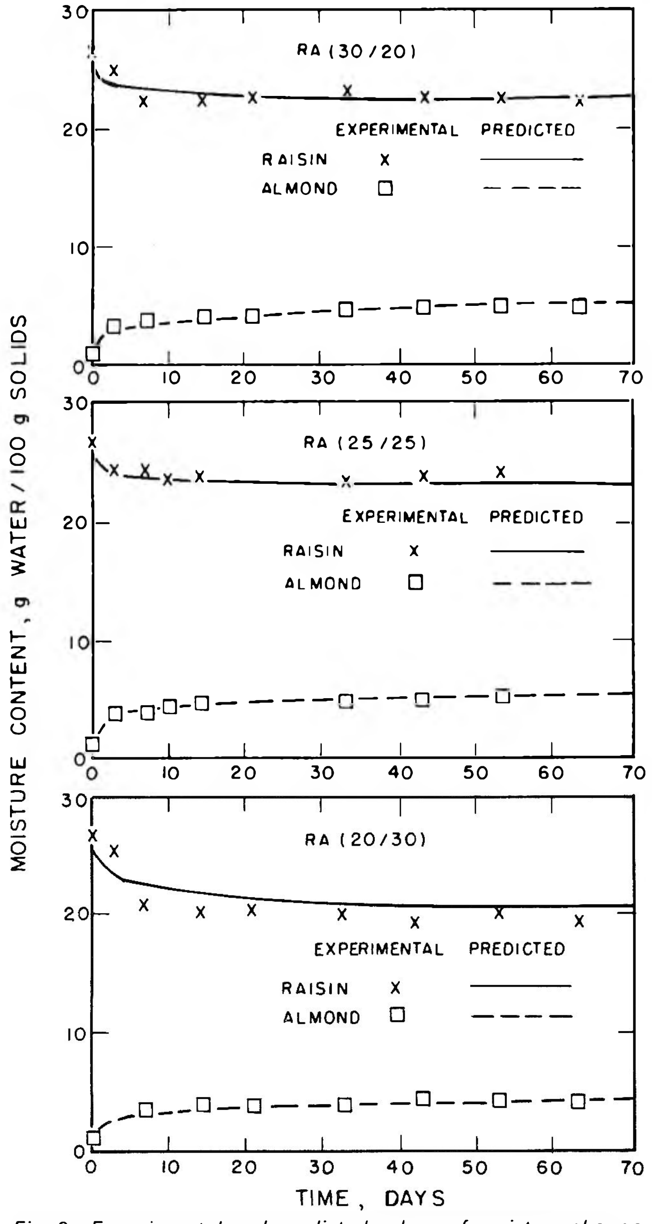

Fig. 2—Experimental and predicted values of moisture change of raisins and almonds in two component mixed systems.

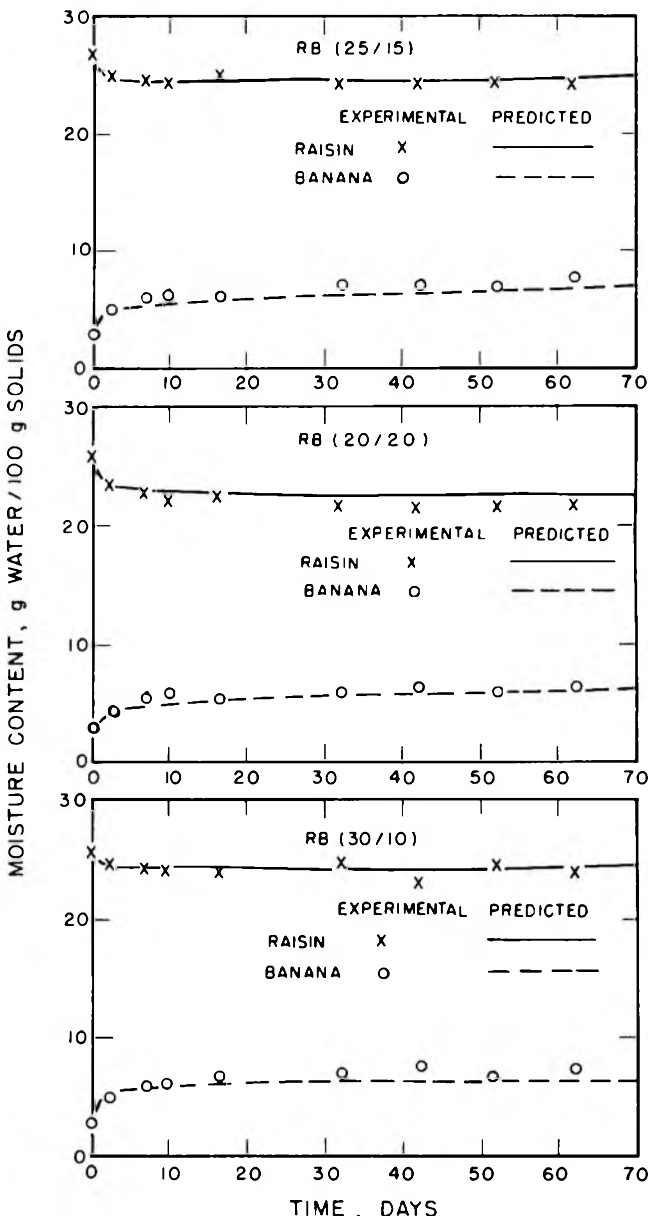

Fig. 3—Experimental and predicted values of moisture change of raisins and banana chips in two component mixed systems.

where M_i is the initial moisture content of the RL component.

The boundary condition is:

$$M(r, z, t) = M_{eq(t)}, \ t > 0 \qquad (5)$$

r, z on the surface,

where the M_{eq} is the moisture content of the rate-limiting (RL) component at the equilibrium water activity of the non-rate-limiting (NRL) component(s). The M_{eq} value was calculated by using mass balance and the Guggenheim-Angerson-DeBoer (GAB) isotherm equation:

$$\frac{M_{eq}}{M_o} = \frac{C\,k\,a_w}{(1 - ka_w)(1 - ka_w + Cka_w)} \qquad (6)$$

where M_o = monolayer of absorbed water, C = constant related to enthalpy of adsorption on monolayer, and k = a constant related to the enthalpy of adsorption for the multilayers as compared to the monolayer.

To determine the M_{eq} for a two-component system, first, the mass balance equation was used to calculate the moisture content of the NRL component. Then the GAB equation was used to calculate the water activity of the NRL component at this moisture content. Then, the GAB equation was used again to calculate the M_{eq} of the surface of the RL component at this water activity. For three or more components, the equilibrium water activity (a_{weq}) of the NRL components were determined by checking the total amount of water of these components at different a_ws. The a_{weq} was assumed to be the a_w when the total amount of water was consistent with the total amount of water at zero time. The flow chart for this computer procedure is shown in Fig. 1. The M_{eq} value was then calculated by using this a_{weq} and the GAB equation. One of the important assumptions in our study is that the surface of the rate-limited component is instantaneously in equilibrium with the NRL components at each time increment, and that the moisture content of each NRL component is considered uniform during each increment. A similar assumption was used by Lomauro and Bakshi (1985), and Chhinnan and Bakshi (1985) where they assumed that the surface moisture content was constant and in equilibrium with the environment during the drying and storage process. However, in our study the surface moisture content was not a constant as it changed during each increment.

The Finite Element procedure used in this study was de-

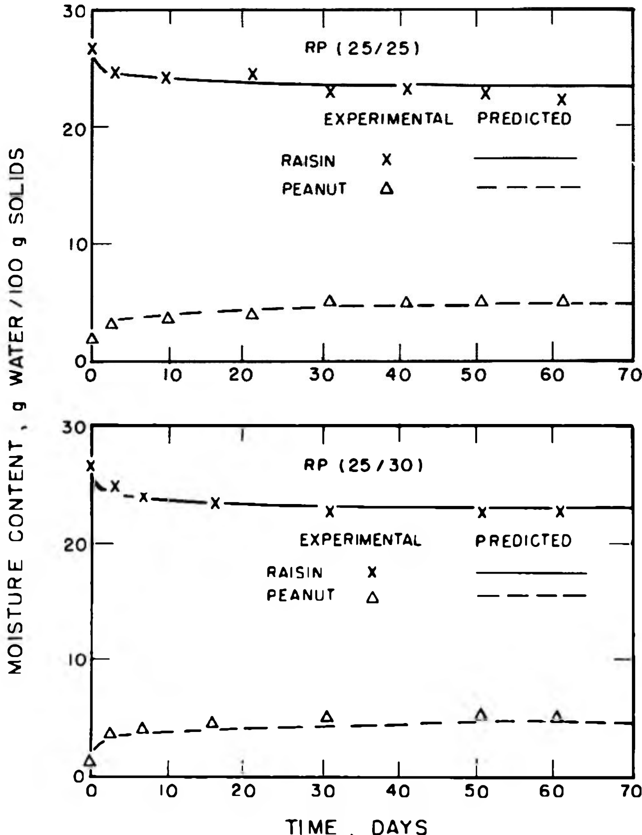

Fig. 4—Experimental and predicted values of moisture change of raisins and peanuts in two component mixed systems.

scribed by Lomauro and Bakshi (1985). The resulting matrix differential equation is:

$$[C]\frac{d\{M\}}{dt} + [K]\{M\} - \{F\} = 0 \quad (7)$$

where [C] and [K] are the square coefficient matrixes, {F} is force vector of known values which can be a function of time, and {M} is the unknown moisture content. After determining the moisture content of the RL component at each node, the volume average moisture content of the RL component is calculated by:

$$M_{avg} = (V_e \cdot M_e)/\Sigma V_e \quad (8)$$

The M_{avg} is the volume average moisture content, M_e is the average moisture content of each element and V_e is the volume of this specific element. The total amount of water transferred from the RL component at this time step is:

$$\text{Water} = W_{RL}\{M_{avg}(t) - M_{avg}(t - \Delta t)\} \quad (9)$$

W_{RL} is the weight of the RL component. For a two component system, all water was assumed to be absorbed by the other component. For a three-component system, this amount of water was absorbed by each NRL component at a different rate. The ratio of water absorbed by each NRL component is assumed to be the ratio of weight times the exponent of rate limiting factor [i.e., $W_{NRL} \cdot \exp(D_{eff}/L_o^2)$]. The surface moisture content of the RL component at the next time step was then calculated based on the equilibrium water activity of NRL components.

The computer program developed in this study was implemented on a CDC Cyber 74 computer. A preliminary computer run showed that 6 hr was an adequate time step increment.

Comparison of predicted values with experimental results

Table 2 shows the moisture transfer properties for each component. The rate factor value (D_{eff}/L_o^2), ranges from 8.9 · 10^{-5} hr^{-1} for raisins to 338.3 · 10^{-5} hr^{-1} for banana chips. This indicates that the raisin is the rate limited component in the system. Based on this, the raisin geometry was divided into 63 nodes and 48 elements for each quarter cross section for the finite element analysis. Because of the symmetry of

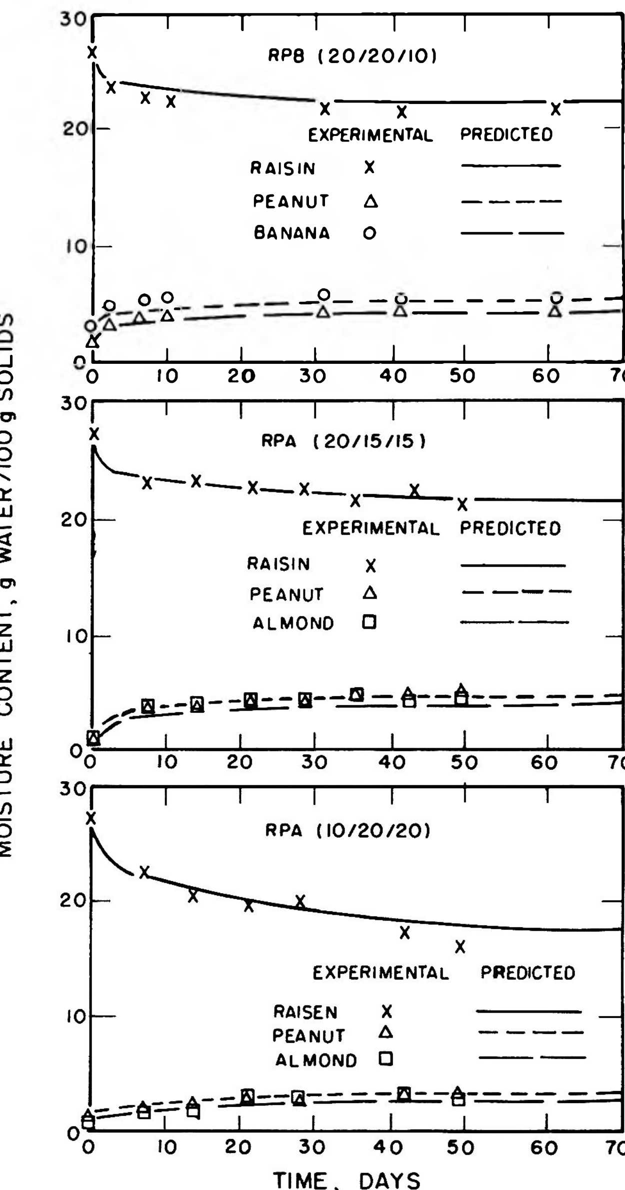

Fig. 5—Experimental and predicted values of moisture change in three component mixed systems.

Table 3—Mean error for moisture prediction and time to reach equilibrium

System[a]	Mean error as percentage of moisture content	Time to reach equilibrium (day)
RP(25/25)	0.48%	7.5
RP(25/30)	0.36%	10
PA(30/20)	0.28%	6
RA(25/25)	0.29%	12.5
RA(20/30)	0.84%	20.5
RB(25/15)	0.42%	3
RB(20/20)	0.47%	8.5
RB(30/10)	0.48%	1
RPB(20/20/10)	0.62%	14.5
RPA(20/15/15)	0.42%	19
RPA(10/20/20)	0.52%	52

[a] R = raisin; P = roasted peanut; A = roasted almond; B = banana chip (a/b/c): weight of each component in grams

this quadrant, only one-half of the raisin geometry was analyzed for the Finite Element model.

The experimental results of weight gain versus time for each component mixture are shown in Fig. 2 to 5. It is obvious that two stages of moisture transfer take place. The first stage shows a rapid moisture gain within the first week while the second stage shows a very slow increase for the rest of the time.

The lines in Fig. 2 to 5 are the volume average moisture contents for each component as predicted by the model. The mean error for each study is shown in Table 3. The range of mean error is from 0.28% to 0.84% for the two-component system, and 0.42% to 0.62% for the three-component system. Thus, the predicted values were found to be in satisfactory agreement with experimental results.

The time to reach equilibrium (T_{eq}) is also shown in Table 3. The range of T_{eq} is from one day for RB(30/10) to 52 day for RPA(10/20/20). Comparing the packages with a 1:1 ratio of components, the T_{eq} for RA(25/25) is 12.5 days, the T_{eq} for RB(20/20) is 8.5 days, and the T_{eq} for RP(25/25) is 7.5 days. In the model, T_{eq} is a function of the rate factor of the RL component, and not a function of rate factor of NRL component. The T_{eq} is also a function of amount of water needed to transfer before equilibrium, and the isotherm properties for both the RL and NRL components.

In spite of the good agreement between the predicted and experimental results, this model has several assumptions which may not be suitable in some cases. The diffusivity value may have a moisture content dependency as is found in drying; the initial moisture content may not be uniform; and the size of component may change with time (Lomauro and Bakshi, 1985; Zhang et al., 1984) if shrinkage occurs. However, the change in properties may be overcome by modifying the Finite Element program for each node.

NOMENCLATURE

a_w	water activity
a_{wbest}	best water activity in calculation loop used in Figure 1
a_{weq}	equilibrium water activity
$a_{whighest}$	highest water activity used in Fig. 1
a_{winv}	interval of water activity defined in Fig. 1
$a_{wlowest}$	lowest water activity used in Fig. 1
C	Guggenheim constant
[C]	coefficient matrix
D	diffusion coefficient
D_{eff}	effective diffusion coefficient
{F}	force vector
H	moisture difference value
k	factor correcting properties of the multilayer molecules with respect to the bulk liquid
[K]	coefficient matrix
L_o	characteristic length
M	moisture content
M_{avg}	volume average moisture content
$M_{avg(t)}$	volume average moisture content at time t
M_a	actual moisture content
M_e	average moisture content of each element
M_{eq}	moisture content of RL component at the equilibrium water activity of NRL component(s)
$M_{eq(t)}$	equilibrium moisture calculated at time t
M_i	initial moisture content of RL component
M_o	monolayer value
M_p	predicted moisture content
M_T	moisture content at time T day
M_{T+13}	moisture content at time T + 13 day
m	total number of data points
n	total number of components
N_{ewTw}	total amount of water at different a_ws used in Fig. 1
NRL	nonrate limiting
r, R	radius
RL	rate limiting
T, t	time, days or second
t_{stop}	total calculation time used in Finite Element program
T_w	total amount of water used in Fig. 1
V_e	volume of each element
W_{NRL}	weight of solids of NRL component
W_{RL}	weight of solids of RL component
z	length
α	difference between T_w and N_{ewTw} used in Fig. 1
β	smallest α value in a loop used in Fig. 1
γ	calculation loop indicator used in Fig. 1

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Production of Apple Juice by Single Pass Metallic Membrane Ultrafiltration

R. L. THOMAS, P. H. WESTFALL, Z. A. LOUVIERI, and N. D. ELLIS

ABSTRACT

A new process utilizing single pass metallic membrane ultrafiltration (UF) was developed to produce apple juice. Pureed whole apples were treated with cellulase and pectinase at 50°C for 2 hr, resulting in a 60% decrease in viscosity. To obtain clarified juice, enzyme treated puree was pumped through a single-pass, tubular membrane system consisting of a metallic oxide membrane formed-in-place on the porous structure of sintered stainless steel tubes. Juice yields of 80–85% were obtained with a single pass, and gas chromatographic profiles of ultrafiltered juice were nearly identical to that of the original apple puree. Permeabilities and sugar rejection characteristics of the UF system were dependent on tube diameter.

INTRODUCTION

ULTRAFILTRATION (UF) has been used for the clarification of pressed, prefiltered apple juice (Heatherbell et al., 1977) and other pressed fruit juices (Kirk et al., 1983). The permeate flux of hollow fiber or thin-channel polymeric membrane systems declines appreciably during operation due to membrane fouling, which becomes more pronounced as feed concentration increases (Breslau and Kilcullen, 1977). Also, polymeric membranes are subject to compression effects as transmembrane pressure is increased, resulting in further reduced flux rates (Kirk et al., 1983). Thus, large pressure drops across hollow fiber or thin-channel ultrafiltration membranes cannot be achieved, necessitating the use of extended recirculation or diafiltration. These ultrafiltration systems can only be applied to the clarification of pressed, prefiltered juices. Even with pressed juices suspended solids can cause blockage of the flow channels, and severe membrane fouling can occur due to concentration effects as a result of recirculation.

Open tubular UF designs offer the advantage of handling solutions high in suspended solids and viscosity due to large, circular flow channels. A disadvantage to this design is the relatively low membrane packing density and the high cost of the tube support needed for higher pressure operation (Paulson et al., 1984). However, if open tubular systems can be operated in a single pass the best possible average flux can be maintained (Brandon, 1985).

The purpose of this study was to determine if ultrafiltered apple juice could be produced in a single pass operation by pumping high solids apple puree directly through a large diameter tubular membrane system composed of sintered stainless steel tubing with a formed-in-place metallic UF membrane.

Materials & Methods

Apple puree

A blend of freshly harvested Red Delicious and Golden Delicious (3:1) apples was provided by Carolina Products, Inc. (Greer, SC). Whole apples were comminuted in a Fitz Mill with a 65 mil screen. To reduce viscosity, the apple puree was treated with both ENZECO pectinase and cellulase (Enzyme Development Corporation, New York) at equal concentrations of 0.002% enzyme based on total weight of apple puree.

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Treatment was for 2 hr at 50°C with constant stirring. In some cases the puree was formed by grinding whole apples in a hammermill, enzyme treating the pulp as above, and then finishing to remove seeds and skins. Both methods resulted in purees of similar properties. Relative viscosity measurements were made with a Brookfield Synchroelectric viscometer.

Metallic membrane ultrafiltration

The metallic membrane ultrafiltration system was provided by Carre, Inc. (Seneca, SC). In most cases, the system consisted of 5/8 inch (i.d.) × 140 ft (1.56 cm × 42.67 m) porous sintered stainless steel tubes, although 1-¼ inch (i.d.) × 10 ft (3.12 cm × 3.04 m) tubes were also studied. The porous sintered tubes were housed within a stainless steel shell which collected the permeate as it passed through the membrane by outward flow. The metallic oxide membrane (ZOSS) was formed-in-place on the inner surface of the sintered stainless steel tubes. Additional, details of ZOSS membrane formation are proprietary information (Brandon, 1985). The system was typically operated as a recirculating 20 gal (75.7 L) batch system, shown schematically in Fig. 1. Apple puree or sauce from the comminutor (A) was placed in a steam-jacketed kettle (B) for enzyme treatment. The kettle also served as a temperature controlled feedtank to maintain the puree at 50°C throughout the experiment. The puree was pumped from the kettle by a centrifugal suction booster pump (C) to a diaphragm pressurizing pump (D), which fed the puree to the ultrafiltration module (E). Concentrate (retentate) flow (2) from the module was controlled by a small gear pump (F), which also served as a pressure control valve. Pressure at the inlet was controlled by a manually operated by-pass valve (V_3). The by-pass line (4) was returned to the feedtank (B). Pressure gauges were installed at the feed stream (1) inlet and in the concentrate stream (2) outlet to determine inlet-outlet pressure differential (pressure drop). Inlet pressures ranged from 300–1000 psi (2,070–6,400 kPa) and outlet pressures ranged from 500–50 psi (3450–345 kPa). Permeate (3) flux measurements were made by the stop-watch and graduated cylinder method and expressed as gallons/ft^2 membrane/day (GFD). (1 GFD is equal to 1.698 L/m^2/hr). Membrane permeability was expressed as permeate flux (J) divided by pressure (P). Juice yield was determined from the mass balance of permeate and concentrate produced over a 10 min period. The membrane was cleaned-in-place with a base/acid wash, which consisted of 0.015% sodium carbonate (pH 9.0) followed by a 0.02 M ammonium acetate buffer, pH 4.8, with intermittent flushes with hot water.

Analysis of voltiles

The volatiles of untreated apple puree, enzyme treated puree, and ultrafiltered juice were analyzed by gas liquid chromatography using a Hewlett Packard 5880-A gas chromatograph equipped with a flame ionization detector and a 5880-A series level four computerized data system. The column was a 50 meter Carbowax 20 M fused silica capillary column. Puree samples were suction filtered on a sintered glass filter and injected directly onto the column. Sample preparation may have caused some reduction in the most volatile components of the puree, but the effect was considered to be negligible. Ultrafiltered juice was injected directly without pretreatment. Sample size was 5μL split 15:1 and helium was used as carrier gas with a flow rate of 1 mL/min. The oven temperature was maintained at 60°C for 7 min before increasing to 200°C at a rate of 5°C/min and holding for 25 min. Total volatiles were determined by summation of the integrated peak areas from the chromatograms and percent area under each peak was calculated.

Sugar analysis

Total sugar concentration was measured by refractometry. Individual sugars were analyzed by high performance liquid chromatography

METALLIC MEMBRANE ULTRAFILTRATION SYSTEM FOR APPLE JUICE CLARIFICATION

APPLES
PUREE
A
STEAM IN
CONDENSATE OUT
B
T
V_1
V_2
V_3
C
D
F
P
E
1
2
3
4

A - COMMINUTOR
B - CONSTANTLY STIRRED JACKETED STEAM KETTLE
C - CENTRIFUGAL SUCTION BOOSTER PUMP
D - DIAPHRAM PUMP
E - METALLIC MEMBRANE UF UNIT
F - GEAR PUMP

1 - FEED STREAM(ENZYME TREATED APPLESAUCE)
2 - CONCENTRATE STREAM
3 - PERMEATE STREAM
4 - BYPASS STREAM
V_1 - MANUAL CONTROL VALVE
V_2 - AUTOMATIC CONTROL VALVE
V_3 - MANUAL CONTROL VALVE

Fig. 1 — Flow diagram for single pass, metallic membrane ultrafiltration of apple puree.

(HPLC) using a Beckman Model 110A HPLC equipped with an LDC differential refractometer and an Altex model C-RIA integrator. Sugars were separated on a Bio-Rad Bio-Sil Amino 5S carbohydrate column eluted with acetonitrile/water (85/15) at 25°C. Solutions of 1% fructose, sucrose, and glucose were prepared as standards for identification and quantification by the external standard method. Puree and juice samples were prepared for analysis of volatiles as described above except that the juice samples were passed through a 3 ml Baker-10 SPE C_{18} column prior to injection.

RESULTS & DISCUSSION

FOR THIS STUDY a large diameter, tubular UF membrane system composed of sintered stainless steel tubing with a formed-in-place metallic membrane was constructed. Formed-in-place refers to the process of forming the membrane remotely after the support tube system has been assembled by passing a feed solution containing the membrane forming material tangential to the porous surface of the support at high velocity under pressures from 500–1200 psi (Marcinkowsky et al., 1966). This membrane formation has been described using natural polyelectrolytes, synthetic organic polyelectrolytes, and inorganic hydrous oxides (metallic membranes) (Thomas, 1977). Since large pressure drops could be achieved with the rigid stainless steel backing and high solids feeds could be used with the large diameter flow channel, this system appeared to be uniquely suited for the production of clarified juice from comminuted apple puree in a single pass operation.

Treatment of apple pulp with a combination of cellulase and pectinase effectively reduced viscosity and increased yields of free-run juice (Kilara, 1982). Since a reduction in apple puree viscosity prevented excessive pressure drop between feed inlet and concentrate outlet and allowed for greater juice recoveries and less membrane fouling in a single pass system, the feed was pre-treated with cellulase and pectinase to reduce viscosity as much as possible. On the average, the enzyme treatment resulted in a 55–65% reduction in viscosity of the purees. This was accomplished with a 2 hr treatment at 50°C, which approximated the pectinase treatment period for pressed apple juice currently used in apple juice manufacturing.

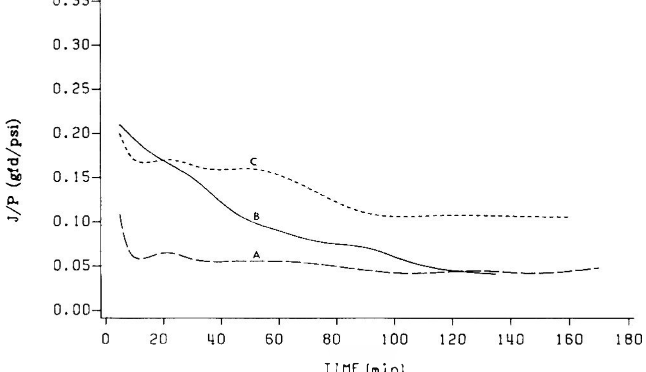

Fig. 2 — The effect of high solids apple puree on the fouling characteristics of ZOSS metallic membranes: (A) 5/8 in. (1.56 cm) tubes at 65% yield and 300 psi pressure drop; (B) 5/8 in. (1.56 cm) tubes at 15% yield and 100 psi pressure drop; (C) 1-1/4 in. (3.12 cm) tubes at 12% yield and 50 psi pressure drop. Permeability expressed as flux (J) divided by pressure (P). Flux given as gal/ft²/day or gfd and pressure as lb/in² or psi.

The effect of high solids apple puree on the permeability characteristics of the ZOSS metallic membrane is shown in Fig. 2. Curves A and B represent membrane fouling for 5/8 in. (1.56 cm) tubes and curve C represents fouling for 1-¼ in.

Table 1 — Effect of tube diameter and juice yield on sugar rejection during ultrafiltration

Tube diameter (in./cm)	Juice yield (%)	°Brix Apple puree	°Brix Ultrafiltered juice	Sugar rejection (%)
5/8 / 1.56	15	12.0	11.0	8.3
5/8 / 1.56	65	11.6	10.0	13.8
1¼ / 3.12	12	10.5	10.5	0.0

(3.12 cm) tubes. Membrane fouling was determined by measuring changes in permeability (J/P) over time. Membrane fouling occurred more rapidly with the 5/8 in (1.56 cm) tubes than with the larger, 1-¼ in (3.12 cm) tubes. Also, membrane fouling in the smaller diameter tubes was more pronounced at low feed velocity and high juice recovery (curve A) than for high feed velocity and low juice recovery (curve B). Membrane permeability at high feed velocity/low juice recovery eventually was reduced to the steady level obtained very rapidly at low feed velocity/high juice recovery. This was expected since higher feed velocities provides a sweeping action which retarded the build-up of foulants at the surface of the membrane (Setti, 1976). Fouling was much less pronounced for the larger diameter tubes (curve C) operating at high feed velocity and low juice recovery, and the steady state permeability was higher than for the smaller diameter tubes. Large diameter tubes of sufficient length to achieve high juice recovery were not available, but fouling in the large diameter tubes at high juice recovery conditions would be expected to be more rapid initially but stabilize at the permeability level obtained more slowly at low juice recovery as observed with the smaller diameter tubes. High solids apple puree can be ultrafiltered and a steady permeate flux rate can be obtained even at high juice recovery conditions (Fig. 2). Steady state permeability appeared to be highly dependent on tube diameter. Membrane fouling was reversible, since a base/acid wash typically recovered 85–90% of the original permeability to water at 25°C. Steady-state permeability to apple juice was always recovered after cleaning.

In the smaller diameter tubes there was an 8–14% rejection of sugar depending on juice yield, but there was no rejection of sugars in the larger diameter tubes (Table 1). It might be speculated that the foulant layer, which was more evident in the smaller tube membranes was responsible for the partial rejection of sugars. However, this degree of sugar rejection was always observed immediately after start-up and remained constant regardless of the juice recovery and feed velocity conditions even though large changes in permeability were being observed, as during the first hour of operation (Fig. 2). Also, in the large diameter tube membrane no sugar rejection was observed even though permeability was declining during the first hour of operation. Sugar rejection was more pronounced in the smaller diameter tubes at low feed velocity/high juice recovery conditions even though membrane permeability was equal to that observed at high feed velocity/low juice recovery conditions after 2 hr of operation. It appears that sugar rejection was more directly dependent on tube diameter and feed velocity than on membrane fouling.

When permeate was not returned to the feedtank to increase the concentration of the feed, there was a further decline in permeability in the large diameter tube (Fib. 3). At a volume concentration ratio (VCR) of 2.0 the permeability of the large diameter tube membrane was reduced to a value approaching the permeability of fouled smaller diameter tubes at a VCR of 1.0 (Fig. 2). Kirk et al. (1983) and Breslau and Kilcullen (1977) showed that permeate flux rate decreased linearly with logarithm of the concentration in the ultrafiltration of pear juice and cottage cheese whey. The viscosity of solutions increased exponentially with increased solute concentrations (Kirk et al., 1983), and high viscosities can result in laminar flow characteristics or channeling (Lonsdale, 1972) with a slower moving

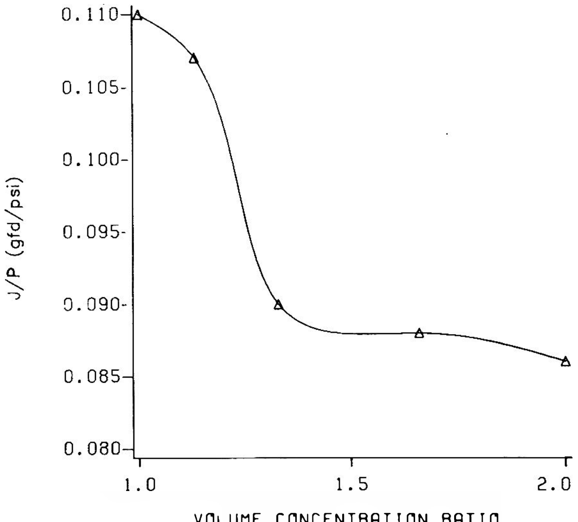

Fig. 3 — Relationship of volume concentration ratio (VCR) and permeability of large diameter (1¼ in./ 3.12 cm) tube membranes.

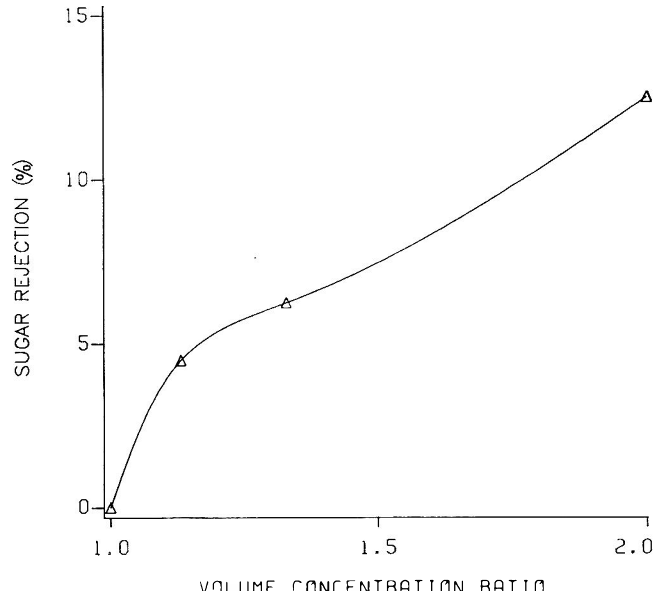

Fig. 4 — Effect of volume concentration ratio (VCR) on sugar rejection of large diameter (1¼ in / 3.12 cm) tube membranes.

layer of feed near the membrane surface. This results in reduced permeate flux even though feed velocities may be only slightly reduced. This slower moving layer at the membrane surface may also be responsible for the observed sugar rejection. As the VCR increased the percent sugar rejection in the larger tube increased even though feed velocity was only slightly reduced (Fig. 4). At a VCR of 2.0 sugar rejection in the larger diameter tube resembled sugar rejection at a VCR of 1.0 in the smaller diameter tubes (Table 1). It appeared that at higher feed solids and viscosity, channeling in the larger diameter tube began to occur. Therefore, the effects of viscosity on flow characteristics with the normal feed may explain the differences in steady state permeabilities and degree of sugar rejection between the large and small diameter tube membranes.

HPLC analysis of the sugars in the ultrafiltered juice from the smaller diameter tube and the original apple puree showed that the ratios of the individual sugars, glucose, fructose, and sucrose were not altered by ultrafiltration even though there was overall reduction of total sugar concentration in the juice (Fig. 5 and Table 2). This indicates that sugar rejection was

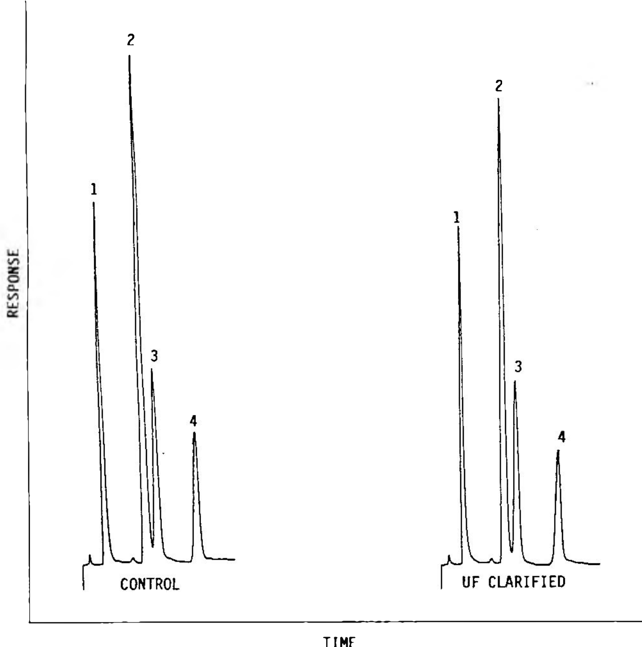

Fig. 5 — HPLC chromatograms of sugars in apple puree feed (control) and ultrafiltered (UF) juice. 1 = solvent peak; 2 = fructose (7.66 min); 3 = glucose (9.19 min); 4 = sucrose (14.19 min).

Table 2 — HPLC analysis of sugars in ultrafiltered apple juice and initial feed puree

	Apple puree (feed)		Ultafiltered juice	
Sugar	% of puree	% of total	% of juice	% of total
Fructose	6.74	56.8	6.09	56.9
Glucose	3.18	26.8	2.89	26.9
Sucrose	1.95	16.4	1.73	16.2
Total sugar (°Brix)	11.87		10.71	

Table 3 — Changes in distribution of apple volatile components as a result of enzyme treatment and ultrafiltration of apple puree

	Peak area (%)		
Flavor component[a]	Apple puree	Enzyme treated puree	UF Clarified juice
1	1.989	0.952	0.245
2	1.166	0.657	--
3	1.998	1.861	2.257
4	1.014	0.998	0.751
5	10.089	8.866	9.055
6	9.200	10.446	9.710
7	2.930	3.210	2.574
8	0.478	--	--
9	0.635	--	--
10	1.131	1.135	1.299
11	0.402	0.500	0.373
12	1.553	1.386	1.199
13	5.633	3.411	4.102
14	--	0.006	0.279
15	0.554	0.519	0.515
16	0.455	0.689	0.699
17	14.326	13.560	13.205
18	1.124	--	1.057
19	1.069	0.959	1.047
20	44.252	49.772	48.904

[a] Numbers correspond to peak numbers from gas chromatography analysis in Fig. 6.

not selective and provides additional evidence that sugar rejection is a consequence of flow characteristics rather than alterations of membrane exclusion properties due to fouling.

Gas chromatography analysis showed that the volatiles of enzyme treated puree and ultrafiltered juice were nearly iden-

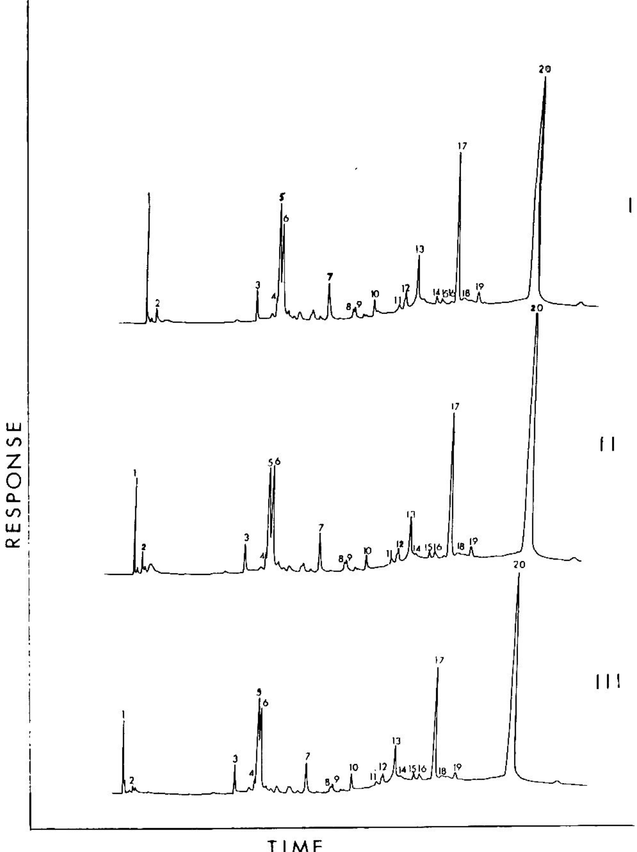

Fig. 6 — Gas Chromatography flavor analysis of original apple puree (I), enzyme treated puree (II), and ultrafiltered juice (III). Total run time was 45 min.

tical to that of the original apple puree (Fig. 6). The total loss of volatiles based on total peak areas from puree and juice was only 14% and the percentage of each flavor component was relatively unchanged by UF operating at 50°C (Table 3). In general, only the most volatile compounds were reduced with a corresponding increase in the proportions of low volatility components. Thus, excellent juice quality was obtained by UF at 50°C, which was the optimum temperature for the enzyme treatment step and also for maximum permeate flux. Permeate flux rate increased as temperature increased up to about 50°C, but higher temperatures did not afford any further improvement in flux rate (Fig. 7).

The juice produced by this process had excellent clarity, which was stable to refrigeration and freezing.

Increasing the pressure drop across the system by reducing concentrate flow rate and thus feed velocity increased the juice yield. Pressure drop increased rapidly at juice yields above 65% with the smaller dimeter tubes (Fig. 8). The pressure drop at 85% juice yield was 700 psi (4830 kPa), and pressure drops above this value were difficult to achieve and still maintain a steady concentrate flow with the smaller diameter tubes. The concentrate at juice recoveries above 85% became very viscous near the outlet end of the UF tube, resulting in a severe pressure drop. Nevertheless, a juice recovery of 85% is more than adequate to demonstrate the commercial feasibility of this process, and larger diameter tubes with sufficient length to achieve high juice recoveries are being investigated to determine if yields greater than 85% can be obtained. Larger diameter tubes should not be as drastically affected by the high concentrate viscosity at high juice yields, and high juice yields could possibly be achieved with lower pressure drops.

CONCLUSIONS

A NEW PROCESS for producing clarified apple juice using ultrafiltration has been described. This process eliminates the

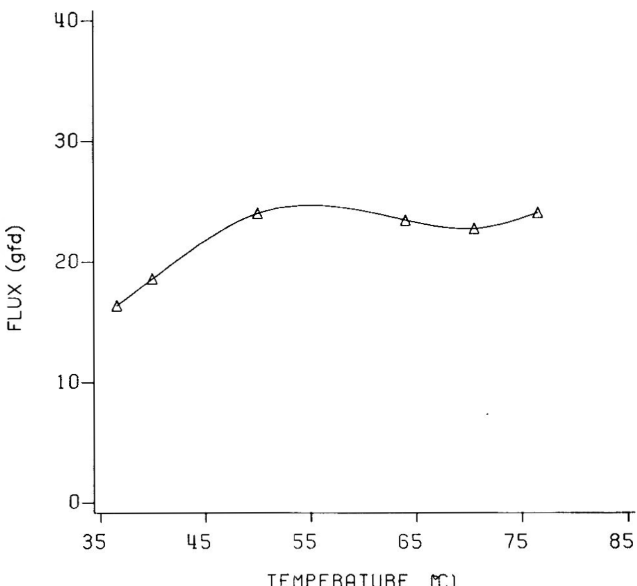

Fig. 7 — The effect of temperature on 5/8 in. tube membrane permeate flux operating at constant feed pressure (300 psi).

need for fruit presses, press aids and prefiltration steps while accomplishing high yield and quality juice by a more direct and simple approach. As with other tubular designs the low membrane packing density and the cost of the sintered stainless steel tube support are disadvantages to this process. However, by utilizing the membrane system as a single pass process the best possible average flux is obtained since all increments of the membrane area are exposed to the minimum possible concentration (Brandon, 1985). This maximizes the average flux that can be achieved when concentration effects are present because there is no recirculation of retentate back to an earlier portion of the membrane. The elimination of pressing and prefiltration equipment and the simplicity and efficiency of a single pass system should offset the higher membrane equipment costs. As with other ultrafiltration systems, this process offers the potential for a higher quality "cold sterilized" juice which does not require conventional heat processing steps.

This study, in general, describes a single pass ultrafiltration system capable of handling high solids feed streams with minimal membrane fouling. This technology should also be applicable to the fractionation of other food products, especially

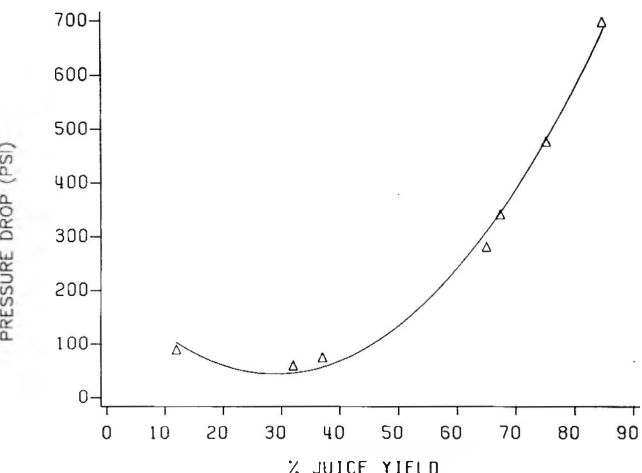

Fig. 8 — Relationship between pressure drop (P_{inlet} - P_{outlet}) and percent juice yields for 5/8 in. tube membranes at 50°C.

those with high solids content and viscosity that are difficult to fractionate by conventional UF technology.

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Influence of Storage on the Composition of Clarified Apple Juice Concentrate

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ABSTRACT

The effect of storage on apple juice concentrate was determined by following changes in composition during a period of 111 days at 37°C. Results showed that storage caused an 87% loss in the total free amino acids, which was mostly due to decreases in glutamic acid, asparagine and aspartic acid. The formol titration method was inadequate for determining the amino compounds involved in Maillard-type reactions. Sucrose was hydrolyzed under these conditions at a rate corresponding to a first order process. The reducing sugars increased at a rate determined by the inversion of sucrose; no consumption attributable to browning reaction was detected. Reduction of organic acids was 9% while apparent phenolic compounds increased from 0.149 to 0.215 g/100g. A maximum accumulation of HMF was observed after 100 days of storage.

INTRODUCTION

DURING STORAGE apple juice is exposed to temperatures which have an adverse influence on quality due to the so-called nonenzymatic browning reaction. Intensive studies of the problem have helped to clarify some of the involved chemical mechanisms and allowed Hodge (1953) to present a lucid, integrated scheme of some of the reactions known to play a role in Maillard-type browning. As knowledge has unfolded, it has become apparent that nonenzymatic browning during storage of apple juice concentrate at relatively high temperatures (Toribio and Lozano, 1984) is highly significant and worthy of study to gain the knowledge necessary to predict and control the color deteriorative process.

In these fruit juices the major constituents believed to be involved in browning are the reducing sugars, amino acids, polyphenols and organic acids (Joslyn, 1956; Cornwell and Wrolstad, 1981). The purpose of the present work was to evaluate changes in the composition of clarified apple juice concentrate during prolonged storage at 37°C. Accumulation of hydroxymethyl-furfural (HMF) was also studied to determine the mode and extent of build-up of this product of hexose degradation under the above conditions of storage.

MATERIALS AND METHODS

APPLE JUICE CONCENTRATE (AJC) (72° Brix, pH = 3.51) was obtained from Ind. Cipoletti S.A. (Cipolletti, Río Negro, Argentina). The juice was stored in aseptic glass vials without head space in the dark at 37 ± 0.5°C and 40–50 mL samples were extracted each week and stored at −20°C until analyzed.

Compositional analysis

Total phenolics were determined using the Folin-Ciocalteau reagent (Singleton and Rossi, 1965). Total acids (titrable acids) were determined by direct titration in accordance with the method reported by the International Federation of Fruit Juice Producers (IFFJP, 1974). Hydroxymethylfurfural (HMF) was also quantitatively determined following the procedure described by the IFFJP (IFFJP, 1974) which is based on the colorimetric reaction between barbituric acid, p-toluidine and HMF.

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Table 1—Changes in total acidity, amino-N compounds, total phenolics and sugars during storage of apple juice concentrate[a]

Storage time days	Acidity g/100g	Formol index meq/100g	Phenolic compounds g/100g	Reducing sugars moles/100g	Sucrose moles/100g
0	2.64	2.85	0.149	0.286	0.0390
7	2.58	2.84	0.153	0.291	0.0376
14	2.47	2.64	0.157	0.290	0.0319
21	2.52	2.68	0.159	–	0.0291
28	2.52	2.57	0.164	–	0.0261
38	2.47	2.57	0.164	0.299	0.0260
45	2.47	2.48	0.169	0.306	0.0267
49	2.41	2.35	0.174	–	0.0233
60	2.41	2.34	0.178	0.313	0.0222
70	2.41	2.27	0.186	0.325	0.0208
84	2.41	2.62	0.196	0.336	0.0180
104	2.35	2.21	0.211	0.332	0.0163
111	2.41	2.35	0.215	0.329	0.0150

[a] Units per 100g apple juice concentrate. Average of two determinations.

Total amino acids were determined according to the formol index (AOAC, 1980). Individual free amino acids were quantified by INTI Laboratories (Miguelete, Buenos Aires, Argentina) with a Beckman amino acid analyzer. (Method: As described in Beckman Manual (118/119 BL/CL IM2, 1977). Analysis of physiological fluids).

Soluble sugars were quantified using a Waters model ALC 244 (Waters Associated Inc., Milford, MA) liquid chromatograph equipped with a differential refractometer R401 Unit, Model U6K injector and Model 6000A solvent delivery system under the following conditions: ambient temperature, refractive index detector, mobile phase acetonitrile/water (80:20) at 1.5 mL/min and chart speed 0.25 cm/min. Samples were diluted (1.000g concentrate to 12.000g) with double distilled water, filtered through Sep-pak C_{18} and Millipore 0.45 μm, injected in 20 μL aliquots and quantified by the external standard method. Since the chromatographic system used in this work was unable to separate glucose from sorbitol, the values of glucose reported here should be interpreted as including sorbitol.

RESULTS & DISCUSSION

Acidity

Results presented in Table 1 show that total acidity as malic acid did not significantly change during storage. The role of organic acids appears to be essentially catalytic (Reynolds, 1965). The slight decrease in acidity might be partly due to copolymerization of organic acids with products of the browning reactions. Lewis et al. (1949) also suggested that organic acids can react with reducing sugars to produce brown pigments.

Total phenolic compounds

Phenolic compounds present in fruit products may react to form brown polymeric compounds (Abers and Wrolstad, 1979). If this reaction plays any role in the color development of apple juice, total phenolics content should not increase during storage as Table 1 shows. Cornwell and Wrolstad (1981) proposed that reductone compounds present in the juices interfere with the Folin-Ciocalteau reagent increasing the apparent phenolics contents.

Table 2—Free amino acid composition of apple juice concentrate. Variation during storage

Amino acid mg/100g conc	Storage time, days						
	0	14	28	45	60	84	111
Aspartic acid	40.7	40.1	35.5	24.2	23.7	22.1	14.8
Threonine	3.1	2.1	2.1	1.84	1.4	1.0	0.6
Serine	9.6	8.8	8.4	7.6	6.4	6.2	4.0
Asparagine	296.8	259.5	233.1	180.3	142.8	140.6	28.7
Glutamine	6.7	2.3	........................trace...				
Proline	3.9	3.6	2.9	2.9	2.9	2.8	1.5
Glutamic acid	57.6	37.1	26.5	16.8	8.2	6.2	2.9
Glycine	0.7	0.6	0.6	0.6	0.5	0.5	0.3
Alanine	4.8	4.7	4.6	4.4	4.3	4.0	2.5
Valine	2.2	2.0	2.0	1.9	.........trace...		
Methionine	1.6	1.5	1.3	0.9	0.7	0.6	0.4
Isoleucine	3.8	3.5	2.9	2.7	1.8	1.8	1.0
Leucine	0.7	0.6	0.5	0.4	0.4	0.3	0.3
Aminc Butyric acid	4.3	3.8	3.3	3.3	2.6	1.6	0.8
Orn,Tyr,Phe,Lys,His,Arg,..........trace...							
TOTAL	438.5	371.2	323.6	247.8	195.8	187.9	57.9
% Retention	100	84.5	73.8	56.4	44.6	42.8	13.2

Amino acids

The amino acids composition of apple juice concentrate is shown in Table 2. The major constituents were asparagine (asn), aspartic acid (asp) and glutamic acid (glu). These values are similar to those found by Burroughs (1957) and Czapski (1975). However, Bielig and Hofsommer (1982), working with about 90 samples of apples, apple juices and concentrates found that every apple juice has a characteristic amino acids spectrum and no mean value can be specified. The other individual amino acids amounted to less than 10%. The concentration changes of total amino acids during storage were very large (Table 2). Asp and glu decreased more markedly than all the other amino acids.

Joslyn (1956) indicated that of the many amino acids present in orange juice lysine (lys) and glu were more likely to be involved in browning. Several studies (Warmbier et al., 1976; Spark, 1969; Eichner and Karel, 1972; Reyes et al., 1982) reported Maillard browning of reducing sugars with only one or two amino acids other than asp, asn and glu. Wolfrom et al. (1974) and Ashoor and Zent (1984) studied the influence of different amino acids in model systems. None of these studies have shown glu and asn to be very high browning producing compounds.

The experimental data were fitted to an exponential equation:

$$AA = 493{,}23 \exp(-0{,}0162\ t) \qquad (1)$$

where: AA = total amino acids content, mg/100g concentrate; $r^2 = 0.986$.

Figure 1 shows the AA value reduction, expressed as percentage of the initial total amino acids content, compared with the nonenzymatic browning (NEB) development, also expressed in a relative way for the same juice at the same conditions (Toribio and Lozano, 1984).

Total amino acids reduction rate (AA_r) can be obtained through the first derivative of Eq (1). Figure 2 shows the AA_r values relative to the initial rate (t = 0) compared with the nonenzymatic browning rate (NEB_r) from previous work (Toribio et al., 1984). Both figures clearly show a significant correspondence between total amino acids content and color development during the storage of apple juice concentrate.

Formol value showed no amino acids consumption during storage. This behavior was also observed by Beveridge and Harrison (1984). While individual amino acids quantification showed that these compounds noticeably diminished, the formol index suggested that only a slight reduction in amino-N occurred during storage (Table 1). It was inferred that formaldehyde reacted with intermediate Maillard compounds masking the actual level of free amino acids.

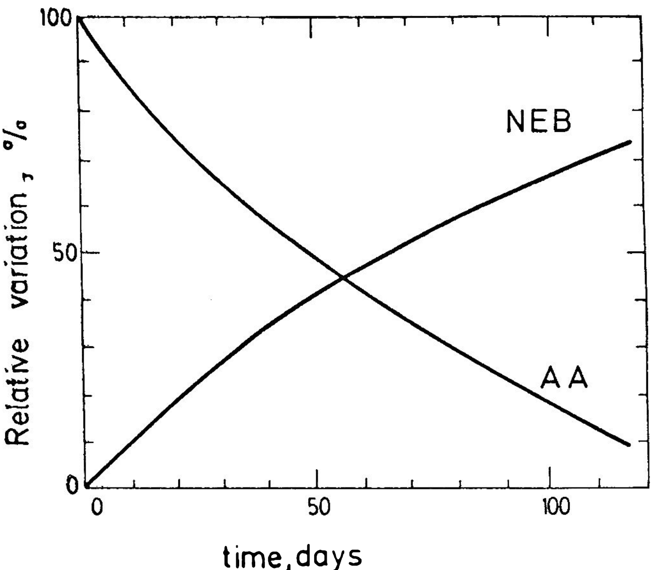

Fig. 1—Extent of total amino acids (AA) disappearance and nonenzymatic browning (NEB) (Toribio and Lozano, 1984) development in apple juice concentrate at 37°C.

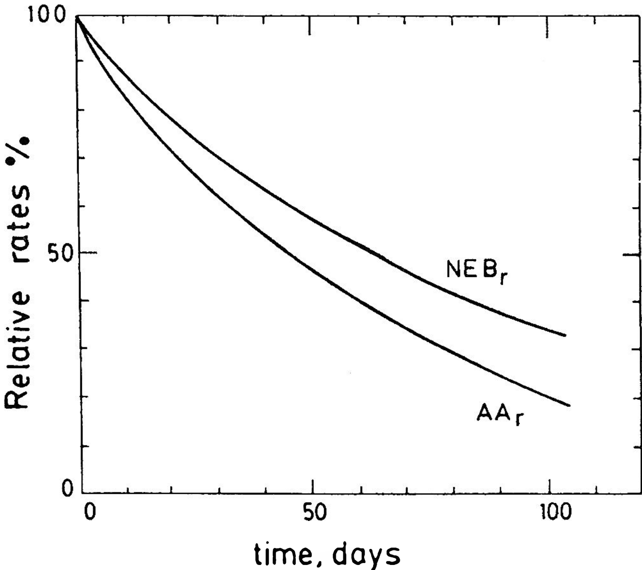

Fig. 2—Nonenzymatic browning rate (NEB_r) and rate of reduction of total amino acids (AA_r) as a function of time of storage.

Carbohydrates

Figure 3 shows the hydrolysis of sucrose after 111 days at pH 3.55 and 37°C. It is well known (Glasstone, 1946) that the rate of hydrolysis is a function of the concentration of reactants, temperature and acid-catalyst concentration. However, if excess water is present the rate of disappearance of sucrose can be represented by a pseudo-first order reaction rate equation:

$$S = S_o \exp(-Kt) \qquad (2)$$

where: S_o = initial sucrose concentration, moles/100g concentrate; S = sucrose concentration at time t; K = rate constant; t = time, min. The experimental data were fitted to this equation, resulting in a value of K = 0.00822 day^{-1}. Hydrolysis, also called inversion because it is accompanied by an inversion of the angle of polarization, yields the two simple

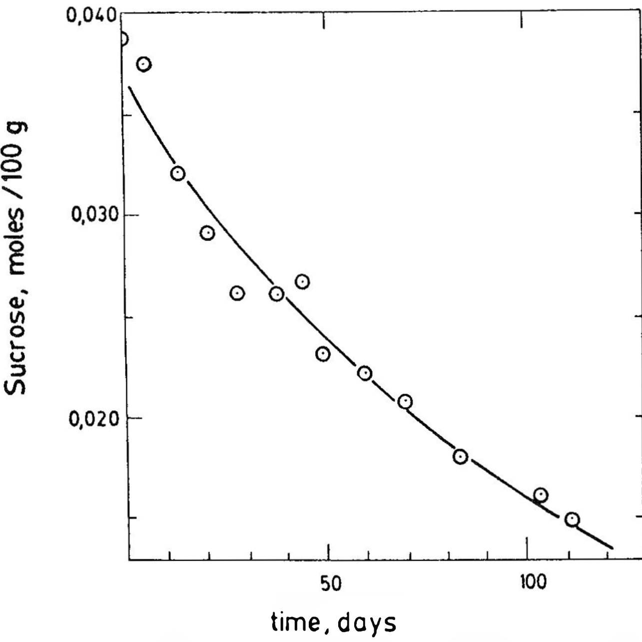

Fig. 3.—Extent of sucrose hydrolysis in apple juice concentrate as a function of time of storage at 37°C: ⊙ Experimental data; (—) Full line represents Eq. (1).

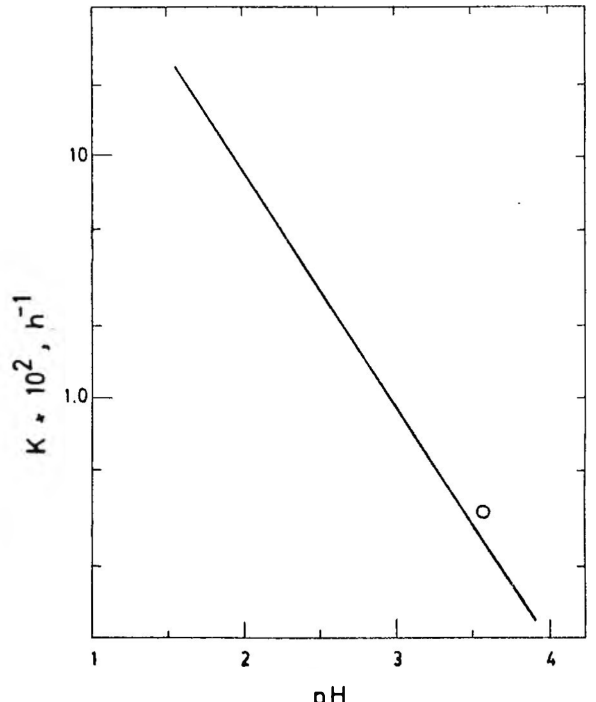

Fig. 4—Dependence of first order rate constant on pH for sucrose hydrolysis at 37°C: ○ Value of K, this study; (—) Full line represents Schoebel et al. (1969) correlated data.

sugars, D-glucose and D-fructose. The rate of appearance of total reducing sugars is described by Eq. (3):

$$R = 2\ S_o\ (1 - \exp\ (-Kt)) + R_o \quad (3)$$

where: R = reducing sugars (glucose + fructose) concentration at time t moles/100g concentrate; R_o = reducing sugar concentration at t = t_o; and t = time, min.

Experimental data obtained by Schoebel et al. (1969) on the dependence of the first order reaction rate on pH, ranged from 1.7–2.76, were fitted to the exponential curve $K = a\ b^{pH}$ (r^2 = 0,996) and extrapolated to pH = 4. Figure 4 compares this information with the K value obtained in this work.

Figure 5 shows the development of total reducing sugars during storage, which increased in concentration in accordance with the predicted kinetics (Eq. 3). Hence, looking at Fig. 3 to 5, hydrolysis appeared to be the major cause of sucrose reduction (and reducing sugars increase) at a rate determined by pH and temperature.

Akhavan and Wrolstad (1980) verified that slight losses (6%) in total sugars occur after 112 days of storage at 37°C of pear concentrate. Stadtman (1948) considered the possibility that relatively small chemical changes are required to produce brown pigment of intense color. If this is the case, the changes in reducing sugars necessary to produce large changes in color might be hard to be detectable. Beveridge and Harrison (1984) detected no loss of reducing sugar after heating a 72.5° Brix pear juice at temperatures up to 80°C for 2 hr. Reyes et al. (1982) found that glucose undergoes more browning than fructose with glycine at 60°C and pH 3.5. Any detectable variation in the fructose/glucose ratio may indicate unbalanced consumption of these reducing sugars due to nonenzymatic browning reaction. No signficant variation was recorded in this study. Mean value was F:G = 1.53 ± 0.05 (w:w) where the range shown is the standard deviation.

HMF

Formation of 5-(hydroxymethyl)-2-furaldehyde (HMF) from amino acids and hexoses or from the acidic degration of hexoses has been widely recognized (Scallet and Gardner, 1945; Reynolds, 1965; Schallenberger and Mattick, 1983). The HMF increase during storage of foods containing hexoses was pos-

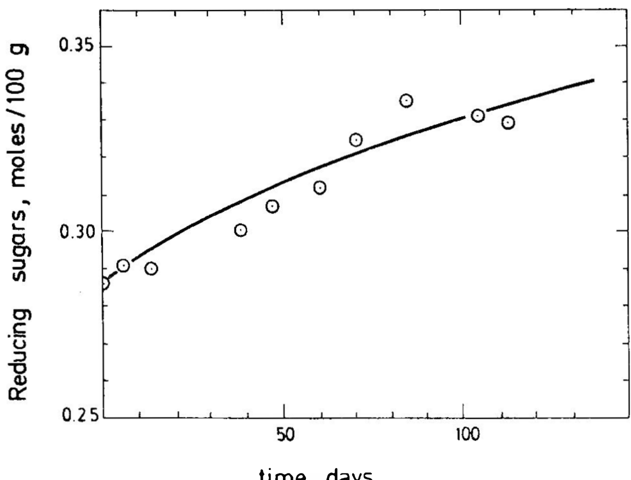

Fig. 5—Increase of reducing sugars as a function of time of storage: ⊙ Experimental data: (—) Full line represents Eq. (2).

itively identified and quantified (Keeney and Bassette, 1958; Drilleau and Prioult, 1971; Resnik and Chirife, 1978).

Figure 6 shows the HMF increase of the concentrated apple juice at 37°C during 111 days of storage. The rate of accumulation can be divided into three period. First period is characterized as an induction time of approximately 2 wk. During the second period the rate showed a rapid increase of HMF with a maximum at 50 days. After that maximum the rate of formation diminished rapidly, and the HMF production approached a plateau of 44 mg/100g concentrate at approximately 100 days. A similar behavior attributable to a second order autocatalytic reaction (Frost and Pearson, 1961) was also recognized by Schallenberger and Mattick (1983) during the acidic degradation of hexoses. It would appear that after 50 days of storage under the present conditions, HMF started to form brown pigments (melanoidins) at such a rate that 40–50 days

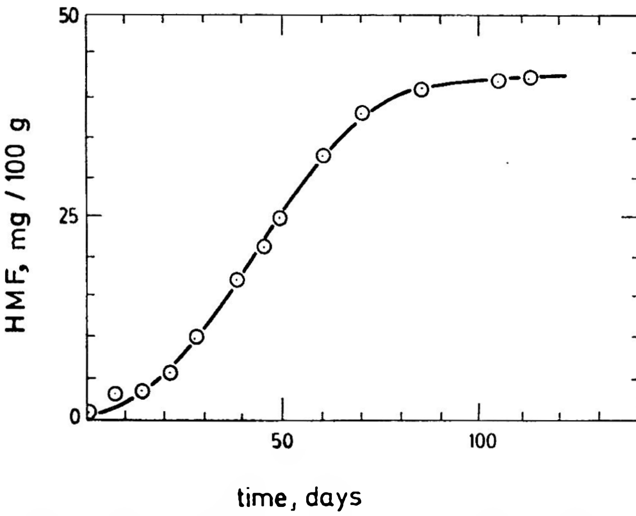

Fig. 6—Effect of storage time on the accumulation of HMF.

later the consumption equalled the formation via Amadori rearrangement of hexose degradation.

SUMMARY

THE CHROMATOGRAPHIC OBSERVATIONS reported in this work indicated that the amino acids present in apple juice were involved in browning reaction. However, results indicated that they reacted at different rates. For example, glutamic acid and asparagine were reduced 20 times and 10 times, respectively, whereas only half of the initial glycine, leucine and proline were consumed during the storage.

Carbohydrate analysis indicated inversion of sucrose under acidic conditions. No loss of reducing sugars due to nonenzymatic browning was detected.

Results of the present study indicate that in an apple juice concentrate properly produced and stored the HMF content is considerably lower than 10 mg/100g but increases with storage and more than 40 mg/100g were found after 100 days at 37°C. Rate of formation of HMF resembled that of a second order autocatalytic reaction which was shown to be characteristic of sugar decomposition. In this case simultaneous formation and consumption of HMF by reaction with other compounds seems to have occurred. It could be inferred that HMF stopped accumulating when the rates of formation and disappearance were equal.

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Determination of Food Preservatives in Orange Juice by Reversed-Phase Liquid Chromatography

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ABSTRACT

An isocratic high-performance liquid chromatographic (HPLC) method has been developed to determine benzoic acid, sorbic acid, and methyl and propyl esters of p-hydroxygenzoic acid (parabens) in orange juice. The HPLC system consists of a reversed-phase column (150 × 6.0 mm i.d.) packed with a rigid polystyrene-divinylbenzene gel, a solvent system of acetonitrile-0.05M KH_2PO_4 (40:60) with flow rate of 1.0 mL/min and 230 nm detection. Including sample preparation, a complete determination can be accomplished in 30 min. The method recovered 93% or more of all preservatives with the exception of high concentrations (500 ppm) of parabens.

INTRODUCTION

BENZOIC AND SORBIC ACIDS are the most commonly used preservatives in fruit juices and soft drinks. The methyl and propyl esters of p-hydroxybenzoic acid (parabens) are frequently used in foods and have potential application for use in combination with sodium benzoate in fruit products and beverages. Extension of the upper pH limit for effective use of benzoic acid can be achieved by the concomitant use of parabens. In addition, parabens are very stable, can tolerate both cold and heat, including steam sterilization (Andres, 1985). Few methods are available for the determination of preservatives in orange juice. An early approach used an ether extraction and UV analysis (Gantenbein and Karasz, 1969); quantitative measurements are based on the strong absorption at 225 nm for benzoic and 250 nm for sorbic acids, respectively. Problems with this method include the presence of interfering compounds, and the broad, intense sorbate absorbance interferes with the benzoate determination (Gutfinger et al., 1976). Bennett and Petrus (1977) separated benzoic and sorbic acids in citrus juices using an anion exchange column.

The existing methods for determining undeclared preservatives are subject to interferences and require extensive sample preparation, gradient elution or lengthy equilibration time which are not convenient for screening many samples. Archer (1980) reported the detection of benzoic and sorbic acid in orange juice using a μ-Bondapak phenyl column and ternary solvent mixture of tetrahydrofuran, methanol and 2% acetic acid. Leuenberger et al., (1979) reported the analysis of sorbic acid, benzoic acid and methyl p-hydroxybenzoate in orange juice using a linear gradient from 20% to 80% methanol in phosphate buffer solution. Fisher (1983) presented an HPLC method for low levels of sodium benzoate in orange juice. Since pulpwash is generally of inferior quality when compared to orange juice, Florida manufacturers of pulpwash are required to add 50–100 ppm benzoate to pulpwash as a tracer to prevent other processors from adding the lower price pulpwash to orange juice. Petrus et al. (1984) using spectral analyses tentatively indicated the presence of undeclared sorbate preservative in some samples of single-strength orange juices from concentrate obtained from the retail market. Since previous methods either were not well suited for routine analysis or could not differentiate between benzoic and sorbic acids, the objective of this study was to develop a simple, reliable method for the simultaneous quantitative determination of sorbic acid, benzoic acid, and methyl and propyl parabens in orange juice suitable for routine analysis.

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MATERIALS & METHODS

Materials

Benzoic acid and sorbic acid were obtained from Matheson, Coleman & Bell manufacturing Co. (Norwood, OH). Methyl and propyl p-hydroxybenzoic acids were obtained from Eastman Kodak Co. (Rochester, NY). A stock 0.1% (w/v) standard solution of all preservatives was prepared by dissolving 1.0g of each standard in 200 mL methanol and then diluting to 1.0L with deionized water. Water was purified by a milli-Q purification system (Millipore Co., Bedford, MA). The HPLC mobile phase consisted of 0.05M KH_2PO_4 (pH 2.65) and acetonitrile (60:40, v/v). It was filtered through a 0.45 μm Durapore filter (Millipore Co., Bedford, MA) and degassed in a sonication bath before use.

Apparatus

The chromatographic system consisted of a Waters Associates (Milford, MA) model 6000A pump, a model U6K injector, model 1202 spectromonitor II (Laboratory Data Control, Riviera Beach, FL). A Shodex RSpak DS-613 column (150 × 6.0 nm, i.d.) was prepacked with rigid polystyrene-divinylbenzene resin (Showa Denko, K.K., Tokyo, Japan) and coupled with a 40 × 3.4 mm RP-18 guard column (Brownlee Labs., Santa Clara, CA). A Hewlett Packard (Palo Alto, CA) photodiode array detection system (Model 1040 A) was used for peak purity determination as described by Miller et al. (1982).

Sample preparation

Six grams of juice were placed into a 50 mL centrifuge tube and centrifuged at 1500 × g for 5 min. A Sep-Pak C18 cartridge was conditioned with 2 mL of methanol followed by 4 mL of water. A 1.0 mL portion of the supernatant juice was pipetted into a syringe and passed through the conditioned cartridge. After washing the cartridge with a 4 mL portion of hexane, the preservatives were slowly eluted with 3 mL of methanol and filtered through a 0.45 μm Durapore filter.

High performance liquid chromatography

Analysis of the preservatives was carried out by injecting 10 μL aliquots of sample or standard onto the column. The preservatives were eluted isocratically with a flow rate of 1.0 mL/min. The effluent was monitored at 230 nm. Chromatograms were recorded using a Soltec model B-281 strip chart recorder (Soltec Corp., Encino, CA) set at 10 mV full-scale. Preservatives were calculated from peak heights using the external standard method.

Recovery test

The recovery of preservatives in juices spiked at three levels (50, 100 and 500 ppm) was determined by dividing each spiked juice into two equal portions. One portion was filtered through a Durapore filter as a control while the other was passed through the Sep-Pak C18 cartridge and a Durapore filter. Recoveries were calculated based on the difference between the total amount determined in the control and the amount obtained after the Sep-Pak clean up.

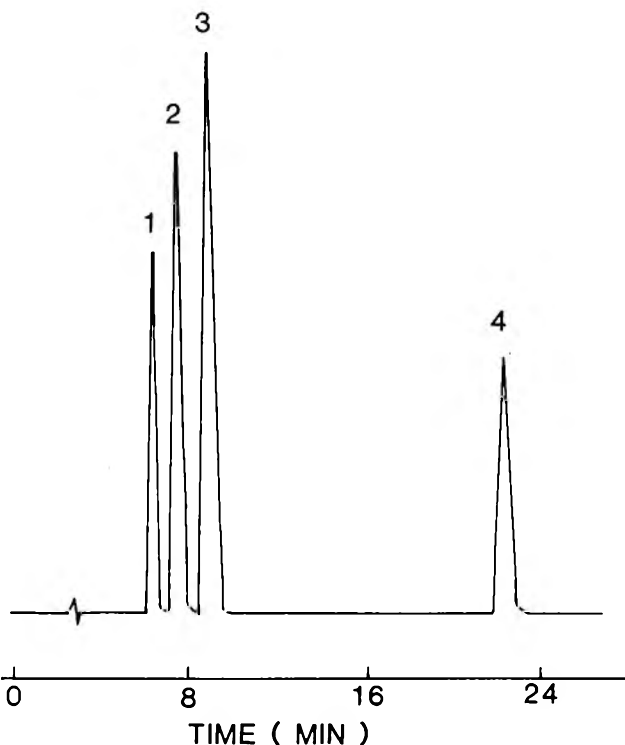

Fig. 1—HPLC chromatogram of standard preservatives. Column: RSpak DS-613; Eluant: 0.05M KH_2PO_4 (pH 2.65)/acetonitrile (60:40, v/v); Flow rate: 1 mL/min; Chart speed: 0.5 cm/min; Peaks: (1) Sorbic acid (12 ppm); (2) Benzoic acid (12 ppm); (3) Methyl paraben (63 ppm); (4) Propyl paraben (63 ppm).

RESULTS & DISCUSSION

Silica based reversed-phase chromatography

A radial-Pak C18 cartridge (Waters Associates, Milford, MA) with 0.05M KH_2PO_4/acetonitrile (80/20, v/v) at pH 4.6 solution was utilized in preliminary studies. Satisfactory separation of the benzoic acid and sorbic acid was achieved, but slight changes in pH significantly affected the resolution of those acids from interfering substances in orange juice. The strong pH dependence of the phosphate buffer on the separation achieved with the C18 bonded silica column limited its application for routine analysis. Therefore, reversed-phase systems using different packing materials were investigated. A possible alternative procedure could involve a column packed with styrene-divinylbenzene resin. This nonpolar material is reported to be more stable toward both low and high pH solvents and has exhibited greater selectivity (α) than the commonly used C18 bonded microparticulate silica packings (Greyson and Patch, 1982; Lee, 1982).

Resin based reversed-phase chromatography

As shown in Fig. 1, base line separation of the four standard preservatives (12-63 ppm) was achieved in approximately 25 min using RSpak DS-613 column packed with polystyrene-divinylbenzene resin. Sorbic acid eluted first followed by benzoic acid and methyl and propyl parabens. Figure 2 shows a chromatogram of orange juice spiked with the four standard preservatives in the range 6–10 ppm. Retention times and calculated resolution for the separation of the preservatives on the RSpak DS-613 column are presented in Table 1. Column capacity factor (K′) for the components of interest should range between 1 and 10 for a multicomponent separation (Karger, 1971). Values ranged from 1.28–6.93, well within the desired range. Resolution values (Rs) were 1.6 or greater.

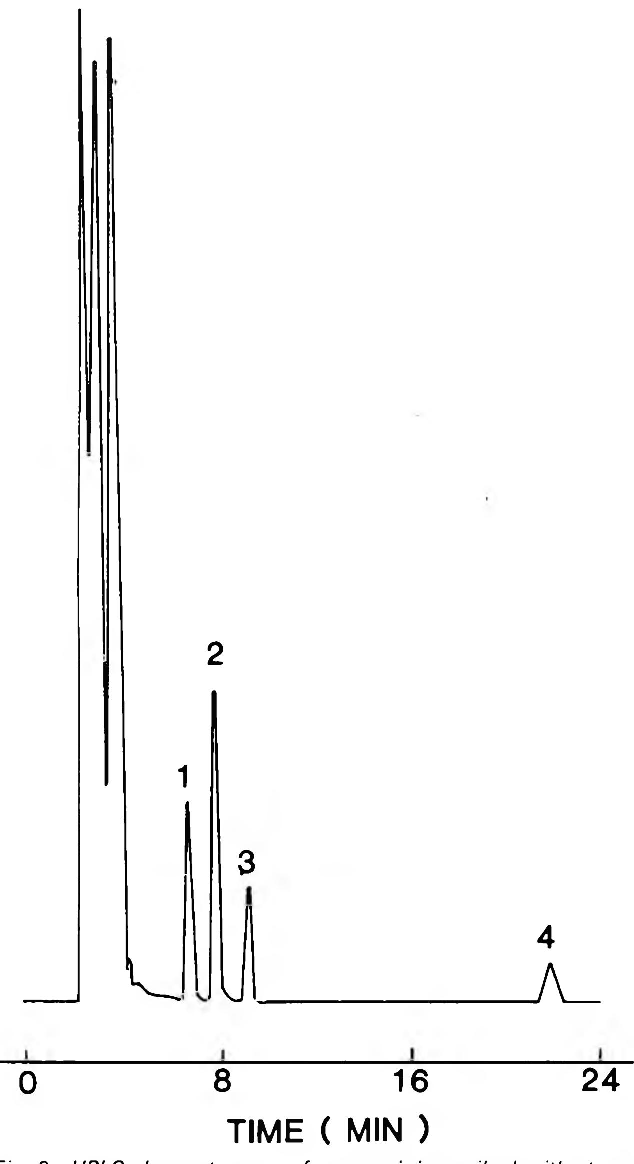

Fig. 2—HPLC chromatogram of orange juice spiked with standard preservatives. Column: RSpak DS-613; Eluant: 0.05M KH_2PO_4 (pH 2.65)/acetonitrile (60:40, v/v); Flow rate: 1 mL/min; Chart speed: 0.5 cm/min; Peaks: (1) Sorbic acid (6 ppm); (2) Benzoic acid (8 ppm); (3) Methyl paraben (10 ppm); (4) Propyl paraben (10 ppm).

Table 1—Retention times and resolution data for separation of preservatives on RSpak DS-613 Column (Flow rate = 1 mL/min; t_o = 2.9 min)

Preservatives	Retention time(min)	CV[a] (%)	K′[b]	α[c]	R_s[d]
Sorbic acid	6.6	2.1	1.28		
				1.27	1.6
Benzoic acid	7.6	1.8	1.62		
				1.36	2.1
Methyl paraben	9.3	1.0	2.21		
				3.14	6.2
Propyl paraben	23.0	2.3	6.93		

[a] Coefficient of variation
[b] Column capacity factor
[c] Column selectivity
[d] Resolution

Detection limits and linear response range

Orange juice blanks consisting of Florida Valencia and Tangelo juices contained no interfering peaks at the retention times where these four preservatives occurred. Detector response was linear for concentrations from 1 ppm to 500 ppm. Average correlation coefficient was 0.98 for the four compounds. The

Table 2—Recoveries of preservatives from orange juice following Sep-Pak clean-up

Preservatives	% Recovery		
	500 ppm	100 ppm	50 ppm
Sorbic acid	95.0	95.8	100.0
Benzoic acid	94.2	96.0	100.0
Methyl paraben	88.4	97.4	95.2
Propyl paraben	85.7	93.8	93.8

selection of the detector wavelength of 230 nm was a compromise made in favor of maximizing the sensitivity for benzoic acid and still detecting all of the other preservatives with reasonable detection limits.

The emphasis on benzoic acid was due to its relative low cost compared to the others. Therefore, it would be expected to be encountered more frequently than the others. Detection limits were approximately 0.2 ppm for benzoic, 0.5 ppm for sorbic and 1 ppm for both methyl and propyl paraben. These detection limits are 10 to 100 times below those commonly used in preservative applications (Chichester and Tanner, 1968). The detection limit can be greatly enhanced for a specific compound by using the appropriate absorption maxima; benzoic acid is 228 nm, sorbic is 259 nm, methyl and propyl parabens are 255 nm in the mobile phase. In fact, sorbic acid could be detected at much lower concentration levels (0.05 ppm) due to its high molar adsorptivity at 259 nm.

Recovery studies

Several workers (Groebel, 1965; Gossele, 1971; Leuenberger et al., 1979) have used direct extraction of the preservatives by an aqueous NaOH solution. Fisher (1983) described a NaOH elution step to remove benzoate from a Sep-Pak C18 cartridge during sample preparation. In our study, this approach gave recoveries for sorbic and benzoic acid over 91% at all levels. However, paraben recoveries were poor, generally, less than 50%. Since there was also interest in parabens, other sample preparation procedures were investigated. The nonpolar packing of the Sep-Pak C18 cartridge was conditioned to retain preservatives of moderate to low polarity dissolved in a polar moving phase, yet reject very polar compounds such as sugar and organic acid in orange juice completely. Recovery rates using methanol to elute the preservatives off the Sep-Pak C18 cartridge from fortified orange juices were very good for all the compounds of interest. Average recoveries of these compounds at all levels were greater than 93% except for the parabens at high concentrations (Table 2). Paraben recoveries were 88.4% for methyl and 85.7% for propyl paraben at the 500 ppm level, respectively. However, it is unlikely that parabens will be found in such amounts due to their relatively high cost and taste problems when used in high concentrations (Chichester and Tanner, 1968).

Survey results

In the application of this method for screening commercial single strength and reconstituted frozen concentrated orange juices for undeclared preservatives, benzoic acid was found in the range 1–212 ppm in 15 samples; 69–190 ppm of sorbic acid was found in five samples. A total of 384 samples from a national survey were tested. One sample showed both benzoic and sorbic acids.

Sorbic and benzoic acid may be added in an amount not exceeding 0.2% by weight in an orange juice and orange juice concentrate prepared for further manufacturing (Anonymous, 1980) if their presence is indicated on the label. The procedure is simple and sensitive. It is most suitable for routine analysis and could be extended to a variety of other fruit juices.

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HPLC Analysis of Organic Acids and Sugars in Tomato Juice

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ABSTRACT

The effect of break temperature on changes in organic acids and sugars in canned juices made from VF145-7879 and UC 82B tomatoes was investigated by HPLC. Oxalic, citric, galacturonic, malic and pyrrolidonecarboxylic acids were shown to be present in the canned juices. Oxalic and galacturonic acids decreased as the break temperature increased. Fructose was present in slightly higher amounts than glucose. Break temperature did not cause significant changes in sugar content of the canned juices made from UC 82B tomatoes.

INTRODUCTION

ACIDITY in tomato juice greatly influences its sensory quality. Organic acids are the main constituents of the acidity. Several reports have been published concerning organic acids in tomato and tomato products (Carangal et al., 1954; Rice and Pederson, 1954; Villarreal and Luh, 1960; Davies, 1964; Miladi et al.., 1969). Luh and Daoud (1971) studied the effect of break temperature on chemical composition of tomato pulp and reported a decrease in titratable acidity when the break temperature was increased. Sherkat and Luh (1976) reported on the quality factors of tomato pastes made at several break temperatures. One of the factors was acidity which was higher in pastes made in lower break temperatures.

Several chromatographic approaches have been published on organic acid determinations, including partition chromatography on silica gel columns with gradient elution (Carangal et al., 1954) and gas chromatographic (GC) determinations of the trimethylsilyl derivatives (Heatherball, 1974). Anion exchange column was used successfully to separate organic acid mixtures (Palmer and List, 1973; Hyakutaki and Hanai, 1975). These methods, however, had some drawbacks, such as low recovery of the acids, incomplete separation and long analysis time. Wilson et al. (1982) applied HPLC for the determination of acids in guava fruit, using a 10 cm radial compression C18 (Waters Associates) column. Good separation and accurate quantitation were reported.

Chromatographic techniques have been used successfully to identify and quantify sugars in food materials. Miladi et al. (1969) used GC to analyze sugars in processed tomatoes. Fructose was present in higher amounts than glucose. Palmer and Brandes (1974) detected glucose, fructose and sucrose in fruits by liquid cation exchange chromatography. Heatherball (1974) reported the separation, identification and quantitation of common fruit sugars by GC as their trimethylsilyl derivatives with a low recovery of the total sugars present. Hyakutaki and Hanai (1975) used high speed liquid chromatography with a 2 meter stainless steel CORASIL AX column for the separation of sugars. Brandao et al. (1980) and Richmond et al. (1981) separated mono- and disaccharides by HPLC using a pair of columns connected in tandem to separate sorbitol from glucose. El Zalaki and Luh (1980) and Martin-Villa et al. (1982) determined sugars in fruits by HPLC using a Micro-Bondapack Carbohydrate column (Waters Associates) with accurate quantitation and short analysis times.

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The objective of this study was to determine the effect of varietal characteristics and break temperature on organic acids and sugars in canned tomato juices by high performance liquid chromatography (HPLC) analysis.

MATERIALS AND METHODS

Tomato juices

VF145-7879 and UC82B tomatoes were grown under controlled conditions on the University farm and harvested at canning ripeness, (fully red, and less than 10% grass green in skin color) washed, sorted, and macerated in a Rietz disintegrator with a 3.175 mm (0.125") screen. The macerates were pumped immediately into a heat exchanger to reach 65, 76, 87, and 104°C, respectively. The holding time was 110 sec for the first three temperatures and 45 sec for the 104°C sample. The hot juices were passed through a Brown juice extractor, filled into #2-1/2 cans, sealed and heat processed in a rotary cooker at 100°C for 10 min. The heat processed juices were cooled in a rotary water cooler at 37°C in 7 min.

Organic acids

A Waters Associates 6000A pump, a U6K Universal injector and a Model R-401 differential refractive index detector were used for HPLC of the organic acids. A Bio Rad Aminex HPX-87 ion exclusion column was used. The tomato juice samples were centrifuged. Ten grams (10 ± 0.02g) of the centrifugate were adjusted to pH 9-9.5 with ammonium hydroxide and passed through a Dowex 1-X8 anion exchange column in the chloride form. The column was washed with four volumes of water to remove sugars and soluble compounds. One milliliter of 10% sulfuric acid was added to the column and the bed was thoroughly mixed. The organic acids were eluted with purified water until 10 mL of eluate were collected. The eluates were filtered through SEP PAK C18 cartridges (Millipore-Waters Chromatography Division, Milford, MA) which were previously saturated with 3 mL of methanol and washed with 20 mL of purified water. Organic acid extracts were filtered through a 0.45 micrometer Millipore filter membrane. The extracts must be free from Ca^{++} ions as indicated by atomic absorption spectrophotometry. A stainless steel column packed with a Bio-Rad microguard ion exchange refill cartridge Bio-Sil NH2 (amino bonded phase) was used. The mobile phase was 0.01N sulfuric acid at 60°C. The flow rate was 0.6 mL per min; the pressure was 1200 psi. Twenty microliters of organic acid extract were injected into the system for each analysis.

Identification of the organic acids was accomplished by comparing their retention time with authentic known acids. Standard curves were prepared for each organic acid in concentrations varying from 0.25 mg/mL to 6 mg/mL. The peak areas of organic acids in the samples were measured and the concentration of each organic acid was calculated from the standard curve.

The organic acids except citric and galacturonic acids were quantitated in the way described above. Galacturonic acid content was determined by the carbazol colorimetric method described by McCready and McComb (1952). The difference between the citric acid-galacturonic acid peak and that of galacturonic acid as determined by the carbazole method represents the amount of citric acid.

Sugars

Sugars (glucose and fructose) in the tomato juices were determined by HPLC analysis. A modification of the method proposed by Richmond et al. (1981) and Martin-Villa et al. (1982) was used. Twenty grams of tomato juice were refluxed with 60 mL of 80% ethanol (high purity) for 1 hr on a water bath at 80°C. The extract was filtered through Whatman No. 54 filter paper. The solids and the residual material in the flask were rinsed with 150 mL 80% ethanol. The combined extracts were concentrated in a rotary vacuum evaporator.

The concentrated extract was made to 25 mL with deionized water and filtered through Whatman No. 42 filter paper. SEP-PAK C18 cartridges (Millipore Waters Chromatography Division) were used to retain the pigments present in the extracts. The cartridges were first saturated with 3 mL of acetonitrile, washed with 10 mL of water and flushed with two volumes of air. The extract was passed through two cartridges and then through a 0.45 micrometer Millipore filter. Thirty microliters of the sample were injected into the Waters Chromatography Division HPLC unit. A stainless steel Micro-Bondapack carbohydrate column 4 mm i.d. × 30 cm (Millipore-Waters Chromatography Division, Milford, MA) and a microguard column 3.8 mm i.d. × 4 cm packed with CO:PELL PAC cyanoamino groups were used for the sugar analysis. Detection of the sugar peaks was based on a R-401 differential refractometer. Isocratic separation of the sugars was achieved at room temperature (22–25°C) using degassed acetonitrile:water (80:20 v/v). The flow rate was adjusted to 1.5 ml/min and the attenuation was set at 16x.

Sugars were identified by comparing the retention times shown in the peaks of the chromatographed samples with those of authentic sugar samples. Calibration curves were prepared for each sugar in concentrations ranging from 1.25 to 20 mg/mL. Quantitation of the sugars present in the samples was obtained from the measured peak areas and the corresponding standard curves.

Statistical analysis

Two samples of each juice were extracted. Analysis were replicated three times on each sample. The data were analyzed for statistical significance by the method described by Welch (1977). The data were evaluated for variance (ANOVA).

RESULTS

Organic acids

Five organic acids were present in the canned juices made from VF145-7879 and UC82B tomatoes. They emerged from the Bio-Rad aminex HPX-87 column when eluted with 0.01N sulfuric acid at 60°C in the HPLC unit in the following order: oxalic, galacturonic, citric, malic and pyrrolidonecarboxylic acids. An example of the chromatograms of the organic acids in the canned juices made from VF 145-7879 tomatoes at four break temperatures is presented in Fig. 1.

Effect of break temperature on organic acids in tomato juices

Oxalic acid was found in larger amounts when the break temperature was at 65°C. It accounted for 22° of the total acidity in the VF145-7879 variety and for 41% in the UC 82B. When the break temperature was raised, oxalic acid in the juice decreased. The sample with a break temperature of 104°C contained no oxalic acid. Since all the canned juices were heat processed at 100°C, the possibility that oxalic acid could be heat labile was discarded. The phenomenon may be interpreted as heat inactivation in the Krebs cycle enzymes at higher break temperatures.

Quantitative aspects of the organic acids

Organic acids in the canned juices as influenced by break temperature are presented in Table 1. Citric acid appeared to be the major organic acid in the canned juice, followed by pyrrolidonecarboxylic acid and malic acid. Galacturonic acid content of the canned juice varied with the break temperature and was highest in the sample made by maceration at 65°C. Since galacturonic acid showed interference with citric acid when eluted from the column in HPLC analysis, it was necessary to determine the galacturonic acid in the sample by the carbazole method.

Pyrrolidonecarboxylic acid

Pyrrolidonecarboxylic acid is a degradation product from glutamine. It may contribute to off-flavor in canned tomato juice (Rice and Pederson, 1954). This acid was found to be present in the canned tomato juices. Pyrrolidonecarboxylic acid

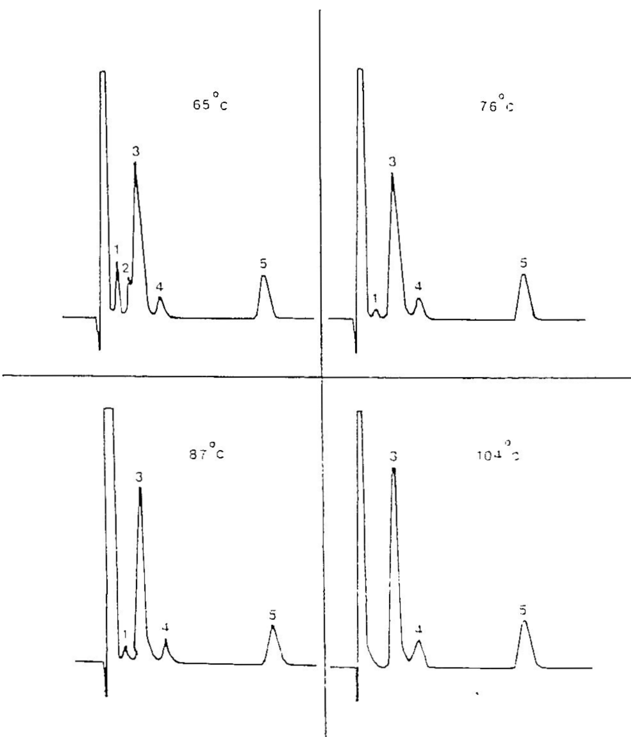

Fig. 1—HPLC determination of organic acid in juices made from VF145-7879 tomatoes at different break temperatures: (1) oxalic acid; (2) glacturonic acid; (3) citric acid; (4) malic acid; (5) pyrrolidonecarboxylic acid.

Table 1—Organic acids in canned tomato juices analyzed by HPLC

Break temp (°C)	Oxalic acid	Citric acid	Galacturonic acid	Malic acid	Pyrrolidone carboxylic acid
	-----mg/100g juice-----				
VF145-7879					
65	115*	31*	226*	40	112
76	13*	200	19*	35	105
87	19*	196	49	37	108
104	0*	350*	0*	49*	120*
UC 82B					
65	207*	82.5*	131*	28	53
76	52*	175*	39	32*	62
87	27*	161	49	24	52
104	0*	163	0*	24	75*

* Significantly different at 95% probability level as determined by SNK test.

(PCA) was higher in juices processed at 104°C. The juices made from UC 82B tomatoes showed a lower PCA content, probably because of a lower glutamine content in this cultivar.

Total acidity

As the break temperature was increased, the total acidity in the canned juices decreased. For the UC 82B variety, a correlation factor of 0.989 was obtained in the regression analysis by least squares method to fit a second degree polynomial equation which described the behavior of total acids when the break temperature was varied. The phenomenon may be explained by the presence of more oxalic and galacturonic acids in juices macerated at 65°C. Simultaneously, galacturonic acid was formed from pectic substances when the break temperature was favorable to polygalacturonase present in fresh tomatoes at 65°C.

Sugars

The elution order of the sugars is presented in Fig. 2. Complete resolution was achieved for the two sugars, and the analysis of each sample was completed in 10 min. Fructose was

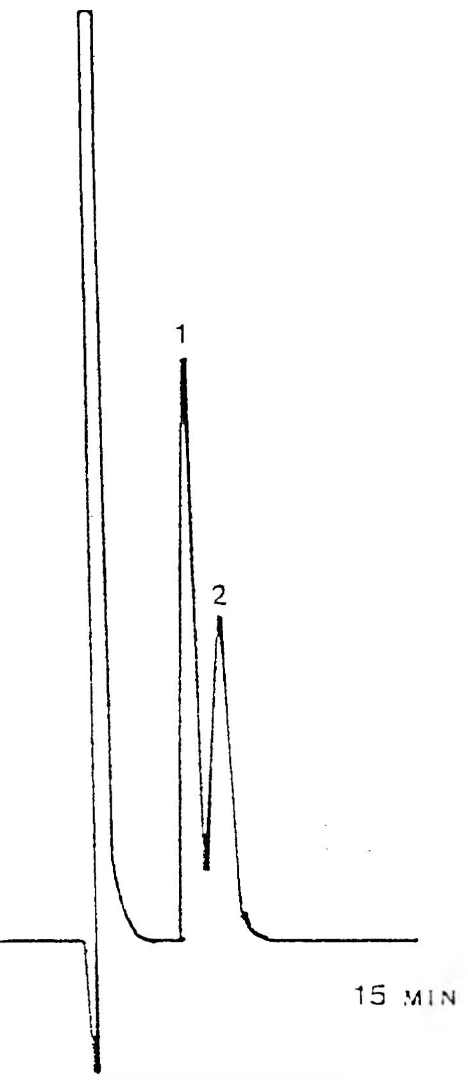

Fig. 2—HPLC determination of sugars in tomato juices: (1) fructose; (2) glucose; Micro-Bondapack-carbohydrate column (Waters); mobil phase, acetonitrile:water, 80:20; flow rate = 1.5 mL/min.; attention 16×.

Table 2—Effect of break temperature on sugars in canned tomato juices by HPLC analysis

Break temp (°C)	Fructose	Glucose	Fructose	Glucose
	g/100g (Fresh basis)		g/100g (Dry basis)	
VF145-7879				
65	1.28*	1.14	23.3*	20.7
76	1.35	1.12	24.5	20.3*
87	1.55*	1.19	28.9*	22.2
104	1.74*	1.39*	26.5*	21.2*
UC82B				
65	1.48	1.24	28.5	23.9
76	1.46	1.27	28.4	24.7
87	1.46	1.25	27.8	23.8
104	1.45	1.26	24.7	21.4

* Significantly different at 95% probability level as determined by SNK test.

found to be more abundant than glucose at all temperatures in both varieties. Martin-Villa et al. (1982) reported on sugar content of various raw and cooked vegetables. They found 1.53% fructose and 1.45% glucose in ripe raw tomatoes. The values for sugars in this investigation (Table 2) were slightly lower than those reported by Martin-Villa et al. (1982). However, the differences were small. It should be mentioned that the sugar content of tomatoes may vary with cultivar, and the fruit yield per acre. Even within the same cultivar, variation in sugar content can be expected due to differences in horticultural practices and seasonal variation.

It was shown that the break temperature in tomato juice processing did not affect the soluble sugar content of the juices made from UC82B tomatoes.

DISCUSSION

ONE POTENTIAL APPLICATION of the HPLC procedure is to supply data to microbiological studies by quantitating bacterial metabolites. Presence of lactic, acetic, propionic and other organic acids caused by microbial growth can be monitored by the HPLC technique.

The HPLC method cannot be used to determine ascorbic acid in tomato juice because as much as 96% was lost when the column was kept at 60°C. The present method can be used to determine organic acids in canned fruit juices. The variation in organic acids attributable to varietal characteristics, processing conditions, microbial contamination, and ripeness levels of the fresh tomatoes can be determined quantitatively. It is a better method than the other methods available today.

The present study illustrated the importance of break temperature on the chemical constituents of canned tomato juices. Varietal characteristics were another important factor. Break temperature was a key variable causing changes in organic acids in the canned juices and will influence greatly the chemical, physical and sensory quality of the canned products.

The separation of serum from the pulp in tomato juices, ketchup and sauces could receive undesirable reaction from the consumer. Careful control of the processing conditions and break temperature would insure favorable consumer acceptance of the canned products.

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Seasonal and Storage Effects on Color of Red-Fleshed Grapefruit Juice

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ABSTRACT

Seasonal and storage characteristics of the juice from four red-fleshed grapefruit (citrus paradisi macf.) cultivars; Ruby Red, Henderson, Ray, and Star Ruby were determined and compared in a search for cultivars having juice with less tendency to fade as the fruit matures and on juice storage. Analyses of the single strength processed juice showed the cultivars to be similar except for lycopene and carotene values. Lycopene, the major pigment in pink and red grapefruit, declined 0.75–0.68 mg/100g, 0.36–0.25 mg/100g, 0.36–0.30 mg/100g and 0.14–0.08 mg/100g for Star Ruby, Henderson, Ray, and Ruby Red, respectively, as the season progressed. Storage of processed juice for 12 months revealed no major decrease in lycopene.

INTRODUCTION

PRODUCERS of Ruby Red grapefruit grown in the United States enjoy a steady fresh fruit market; however, processors encounter a problem in that the pink color of the juice fades during the harvest season taking an an amber hue (Lime et al., 1954, 1956, 1959; Purcell, 1959; Cruse et al., 1979). The problem of beige to amber-colored grapefruit juice has been recognized in red and in white-fleshed grapefruit juice. Other grapefruit cultivars having more intense red flesh are Star Ruby (Hensz, 1971) Henderson (Maxwell and Rouse, 1980) and the Ray (Hensz, 1978). Juice from each of these new red-fleshed cultivars has less tendency to fade in color with increased maturity and juice storage, thus providing the opportunity to evaluate the cause of juice fading. The pink color of the grapefruit is preferred by consumers, thereby making this characteristic economically important. The purpose of this study was to compare the maturity, processing and storage characteristics of the Henderson, Ray, and the Star Ruby cultivars with those of the Ruby Red and determine if the loss of lycopene and carotene could be associated with the development of off-colors.

MATERIALS & METHODS

Grapefruit cultivars

Fruit of the Ruby Red, Henderson, Ray, and Star Ruby cultivars were harvested three consecutive seasons at three intervals during each maturity season. Early-season fruit were picked the second week in December; mid-season fruit the last week in January or the first week in February; and the late season harvest the third and fourth week in March.

Preparation of single strength juice

The fruit were washed on a set of brush rolls and processed as described by Cruse and Lime (1974, 1977). Twenty-four #2 plain tin cans of juice from each cultivar and harvest season were hot-packed at a temperature of 90.5°C, using a variable speed gear pump and a 6.35 mm tubular stainless steel coil pasteurizer. The canned juice was stored in the laboratory's pilot plant at warehouse ambient temperatures (16–36°C). Juice yields for each cultivar at each harvest were obtained from a separate sample of 56 fruit by dividing the juice weight by total fruit weight. Stored processed juice samples were analyzed at monthly intervals. Fresh juice samples from each seasonal harvest were frozen and stored. Analyses were initiated the following day.

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Chemical analyses

Fruit maturity for each cultivar at each harvest, including °Brix, percentage acid, °Brix/acid ratio, pulp (suspended solids) and pH were determined using standard industry procedures (Praschan, 1975). Recoverable oil was determined by the official AOAC procedure (AOAC, 1980). Naringin was determined by the Davis test (Davis, 1947) and vitamin C by the colorimetric procedure of Nelson and Sommers (1945). Juice color was determined on a Gardner Model XL-10 Color Difference Meter using an LR-1 standard. Carotene and lycopene were assayed by the procedure of Lime et al. (1957) using method B. This method determines the absorbance of a hexane extract at 451 nm for carotene and at 503 nm for lycopene. By the use of the specific absorbance of carotene and lycopene, the specific pigment concentration is determined. This method was specifically developed for determining lycopene and carotene in pink grapefruit juice.

Sugar concentrations in stored juices were determined with a Hewlett-Packard Model 1084B high performance liquid chromatograph (HPLC) on a 30 cm × 7.8 mm BioRad HPX-87 Ca^{++} column and detected by differential refractometry. The column temperature was held at 85°C; water was the mobile phase. An HP model 79850B LC terminal/integrator attached to the HPLC was used to quantitate each sugar peak. The HPLC method used is a combination of modified procedures used at this laboratory. The furfural content of stored juices was determined by the colorimetric method of Dinsmore and Nagy (1974).

RESULTS & DISCUSSION

MATURITY ANALYSES on the juice of the four cultivars are summarized in Table 1. All maturity values of the Ray and Henderson cultivars were within ranges previously determined (Cruse and Brown, 1983; Cruse and Lime, 1980). For fresh fruit marketing the °Brix/acid ratio was above the accepted minimum (9–11.5). Juice yield would be expected to increase as the fruit softened with advancing maturity. The juice of the Henderson had a consistently lower acid content than that of the Ray. The oil content of the 'Star Ruby' was consistently lower than that of the three other cultivars.

Pigment analyses on processed juice for early-, mid- and late-season fruit are presented in Table 2. Lycopene values for the Henderson and Ray cultivars were significantly different from either the Ruby Red or Star Ruby fruit and were equal within themselves. Decline of the a/b color ratio was directly correlated with the decline of lycopene.

Carotene values varied; part of this may be due to lycopene conversion and probably a variable carotene content in the juice. Some oxidation may have also occurred. Carotene, however, is not associated with the red or pink color of the juice.

Although the juice color of all cultivars faded as the season advanced, more pink color was retained in the Henderson; Ray and Star Ruby than in the Ruby Red. Thus Ray and Henderson cultivars with their intermediate lycopene content can be included in a blend with Ruby Red juice to enhance the color without rendering the resulting juice or concentrate substandard.

Cruse and Lime (1980) had incorporated 30–40% Star Ruby

Table 1—Maturity characteristics of four grapefruit cultivars for three seasons

Cultivar	Harvest season	Brix	Acid (%)	Brix/Acid Ratio	Pulp (%)	Oil (% x 10^{-3})	pH	Juice yield (%)	Ascorbic acid mg/100mL	Naringin (ppm)
Ruby Red	Early	10.0	1.22	8.3	12.0	7.2	3.3	55.3	29.8	273
Henderson	Early	9.8	1.16	8.5	12.0	7.6	3.3	54.9	28.0	274
Ray	Early	10.1	1.25	8.2	11.0	6.4	3.3	54.7	27.2	248
Star Ruby	Early	10.4	1.21	8.6	12.0	6.0	3.3	54.6	24.6	239
Ruby Red	Mid	10.3	1.21	8.7	14.0	5.2	3.4	55.3	28.8	304
Henderson	Mid	11.0	1.06	9.4	12.0	8.2	3.5	54.7	24.8	344
Ray	Mid	10.2	1.24	8.3	12.0	7.2	3.4	57.2	26.8	269
Star Ruby	Mid	10.1	1.18	8.7	12.0	5.4	3.4	56.6	21.0	288
Ruby Red	Late	10.8	1.16	9.4	13.0	5.4	3.5	58.8	31.0	239
Henderson	Late	9.6	.99	9.6	12.0	6.2	3.6	59.4	24.4	291
Ray	Late	10.3	1.12	9.2	12.0	5.4	3.4	59.5	22.0	255
Star Ruby	Late	10.6	1.11	9.6	12.0	4.5	3.5	56.6	20.4	267
LSD[a]		1.43	0.122	0.67	1.7	1.91	0.18	3.39	6.4	71.3
STD. DEV		1.02	0.097	0.53	1.4	1.51	0.14	2.03	5.1	56.5

[a] LSD - Protected Fisher least significant difference. Any two values within a column which have a difference greater than the LSD are different at $p = 0.05$.

Table 2—Seasonal carotene and lycopene content of processed juice from four grapefruit cultivars for three seasons

Cultivar	Season	Color a/b	Carotene mg/100g	Lycopene mg/100g
Ruby Red	Early	0.41	0.19	0.14
Henderson	Early	0.91	0.36	0.36
Ray	Early	1.02	0.28	0.37
Star Ruby	Early	1.68	0.53	0.75
Ruby Red	Mid	0.34	0.17	0.09
Henderson	Mid	0.77	0.32	0.31
Ray	Mid	0.93	0.27	0.34
Star Ruby	Mid	1.53	0.51	0.68
Ruby Red	Late	0.18	0.16	0.08
Henderson	Late	0.60	0.36	0.25
Ray	Late	0.60	0.25	0.30
Star Ruby	Late	1.27	0.65	0.74
LSD[a]		0.23	0.049	0.063
STD. DEV		0.18	0.039	0.05

[a] LSD - Protected Fisher least significant difference. Any two values within a column which have a difference greater than the LSD are different at $p = 0.05$.

juice in blends with Ruby Red juice and reported an adequate intensity of pinkness in processed juice and storage qualification for acceptance judged by a positive a/b ratio which is considered to be "Grade A" pink grapefruit juice. A negative ratio is considered substandard.

In spite of the insignificant decline of lycopene in the Henderson and Ray cultivars, some brownish tinge was noted, especially in the late-season juice, and to a lesser extent in the mid-season product. This observation indicated that lycopene was not directly involved in the development of the brown tinge observed in processed single strength grapefruit juice. However, color depth was considered deep enough to be marginally acceptable as pink.

Those responses varying at the 0.05 or less probability level by cultivar, season, cultivar and season or not differing by either of these considerations are summarized in Table 3.

The effect of storage on lycopene content in single strength grapefruit juice from early-, mid- and late-harvest are shown in Table 4. The tendency for lycopene to decline was not statistically significant so the *in vivo* decline (Table 2) was more pronounced than *in vitro*. The *in vivo* decline was presumably caused by cyclization of the open-chain structure of lycopene to β-carotene (Britton, 1976). Oxidation by traces of oxygen in the juice and the canning head space may cause the *in vitro* lycopene to level off. Carotene content varied irregularly more than lycopene. Part of this may be due to lycopene conversion and probably to a somewhat variable carotene content in the juice.

In a flavor stability study, Tatum et al. (1975) identified furfural as one of the degradation products formed in canned single strength orange juice during storage. Furfural was also

Table 3—Measured[a] maturity responses varying at the $p \geq 0.05$ level

Cultivar X Season	Season only	Variety only	Neither variety nor season
Acid	naringin	ascorbic	brix
Ratio	pH	acid	pulp
Color	yield		
Carotene			
Lycopene			
Oil			
Pulp carotene			
Pulp lycopene			

[a] Based upon an analysis of variance for each response. There was no significant cultivar by season interactions for any response.

identified as one of the degradation products from ascorbic acid in model studies by Tatum et al. (1969). Earlier model studies by Shaw et al. (1967a, b) indicated both fructose-acid and fructose-base as related to browning in citrus powders. Those studies showed that fructose could be a precursor of the 16 compounds, including furfural, isolated from dehydrated orange powder.

Furfural analysis was made in an attempt to relate the levels of this browning product with the brownish tinge that developed in processed citrus juices on storage at ambient temperatures. Furfural content in processed juice steadily increased during the twelve-month storage period for each harvest season (Table 5). At one month of storage the furfural levels, 80–240 ppb, exceeded the 55 ppb levels that closely paralleled flavor difference in processed orange juice (Nagy and Randall, 1973). The juice of early harvested fruit could be most susceptible to furfural formation, on storage, since there was a 50-fold increase in the browning compound over the 12-month period compared to a 30-fold and 10-fold increase for mid and late harvested fruit, respectively.

The effects of storage on reducing and nonreducing sugar content in the processed juice of the four cultivars are shown in Table 6. The inversion of the sucrose in acid solutions and in canned fruits and juices is well known. Matsumoto (1939) found that 92% of the sucrose hydrolyzed in 144 hr at 30°C and pH 3.37 in an aqueous mixture containing 2% citric acid and 7% sucrose. In this study over 50% of the original sucrose content had decreased by the end of the third month of storage. Sucrose content continued to decline up to the twelfth month of storage. Glucose and fructose content increased and reached a maximum level at ten months of storage. The initial content of reducing and nonreducing sugars was equal, a value that is compatible with results from other grapefruit cultivars (Varsel, 1980). However, the total sugar of the processed single strength juice remained constant over the 12-month period. Therefore, it is unlikely that the reducing sugars, especially fructose, contribute to the brownish tinge that develops in the canned juice during storage as has been observed for citrus powders. Studies

Table 4—Lycopene content of canned single strength grapefruit juice of early, mid and late season harvest

Cultivar	Storage (Months) 0	2	4	6	8	10	12
			Early Season (mg/100g)				
Ruby Red	0.11	0.11	0.09	0.09	0.09	0.08	0.09
Henderson	0.33	0.32	0.33	0.33	0.33	0.29	0.29
Ray	0.32	0.35	0.36	0.36	0.35	0.30	0.33
Star Ruby	0.73	0.74	0.74	0.71	0.71	0.66	0.67
			Mid Season				
Ruby Red	0.07	0.08	0.08	0.07	0.07	0.06	0.07
Henderson	0.26	0.27	0.25	0.23	0.23	0.24	0.25
Ray	0.30	0.30	0.28	0.28	0.28	0.28	0.29
Star Ruby	0.69	0.64	0.64	0.64	0.64	0.64	0.65
			Late Season				
Ruby Red	0.08	0.07	0.05	0.03	0.03	0.03	0.03
Henderson	0.21	0.21	0.18	0.19	0.21	0.21	0.20
Ray	0.27	0.26	0.23	0.23	0.26	0.25	0.25
Star Ruby	0.69	0.65	0.62	0.61	0.63	0.57	0.59

Table 5—Furfural content in canned single strength grapefruit juice

Cultivar	Storage (Months) 1	2	3	4	5	6	7	8	9	10	11	12
						Early Season						
Ruby Red	1.0[a]	1.5	3.0	4.0	8.0	13.5	21.5	24.3	36.8	36.5	46.5	54.0
Henderson	1.0	2.0	3.0	5.8	9.5	15.0	21.5	26.5	44.3	44.0	56.0	60.0
Ray	1.0	2.0	3.0	5.0	7.5	13.0	17.0	23.5	34.3	34.0	48.0	48.5
Star Ruby	1.0	1.5	3.0	5.5	8.8	13.5	22.5	24.0	28.9	39.0	52.0	47.5
						Mid Season						
Ruby Red	0.8	2.2	3.9	6.0	12.0	18.8	22.2	32.8	31.1	44.2	46.9	30.6
Henderson	0.8	2.5	4.2	9.1	13.4	22.6	27.1	38.4	34.3	42.3	44.3	33.4
Ray	1.1	2.2	3.3	6.3	12.0	16.7	18.8	29.7	28.8	32.1	36.3	27.9
Star Ruby	1.0	2.7	4.2	7.3	11.8	19.2	23.7	32.9	30.4	40.3	42.9	33.1
						Late Season						
Ruby Red	2.2	4.4	6.4	10.3	20.5	19.9	30.9	28.2	36.8	29.6	25.0	23.8
Henderson	2.4	5.5	8.5	11.4	23.0	19.9	34.0	33.5	39.6	35.5	29.6	26.4
Ray	1.9	4.3	7.2	9.5	18.8	19.9	28.2	27.6	36.8	26.5	14.6	24.3
Star Ruby	2.4	5.2	7.8	12.5	23.5	23.2	35.5	32.2	44.9	34.9	26.6	29.7

[a] Each mean value × 10^2 represents ppb.

Table 6—Percentage sucrose, glucose and fructose content of canned single strength grapefruit juice from four cultivars

	Month 0	1	2	3	4	5	6	7	8	9	10	11	12
Ruby Red													
Sucrose	4.47	3.62	3.29	1.83	1.37	1.50	—	0.48	0.30	0.16	0.22	0.16	—
Glucose	1.79	1.62	2.10	3.09	3.38	3.48	—	3.55	3.20	4.74	4.42	3.99	—
Fructose	2.31	2.67	2.83	3.47	3.92	4.01	—	4.13	4.35	4.29	4.63	4.38	—
Total	8.57	7.91	8.22	8.39	8.67	8.99	—	8.16	7.85	9.19	9.27	8.53	—
Henderson													
Sucrose	4.20	3.24	3.99	1.82	—	1.36	1.09	1.00	0.32	0.16	0.24	0.05	0.19
Glucose	2.24	1.66	2.74	2.84	—	3.29	3.60	3.54	3.47	4.46	4.35	3.83	4.01
Fructose	2.63	2.65	3.09	3.21	—	3.73	4.24	4.02	4.16	4.18	4.60	4.34	4.26
Total	9.07	7.55	9.82	7.87	—	8.38	8.93	8.56	7.95	8.80	9.19	8.22	8.46
Ray													
Sucrose	4.18	3.92	2.92	1.74	1.46	1.28	1.06	1.10	0.35	0.20	0.23	0.25	0.06
Glucose	2.05	2.00	2.00	2.66	2.77	2.52	3.10	3.12	3.22	3.20	3.60	4.42	3.43
Fructose	2.54	2.23	2.072	2.94	3.23	2.69	3.44	3.49	3.48	3.44	3.74	4.52	3.52
Total	8.77	8.15	7.64	7.34	7.46	6.49	7.60	7.71	7.05	6.84	7.57	9.19	7.01
Star Ruby													
Sucrose	3.81	3.70	3.42	1.91	1.38	1.19	1.11	1.04	0.72	0.23	0.29	0.13	0.16
Glucose	1.72	1.66	1.72	3.10	3.26	2.90	3.23	2.80	3.80	3.38	3.96	3.64	4.16
Fructose	2.27	2.39	1.73	3.73	3.97	3.65	3.83	3.12	4.29	4.21	4.22	4.23	4.54
Total	7.70	7.75	6.87	8.74	8.61	7.74	8.17	6.96	8.81	7.82	8.47	8.00	8.86

by Tatum et al. (1967, 1969) on nonenzymic browning of orange powder showed that furfural formed during acid-catalyzed hydrolysis of ascorbic acid.

The fading of the pink color of grapefruit juice during the season correlate well with decreased lycopene. The brown tinge that developed could possibly be due to a combination of lycopene loss and furfural production in the processed stored juice. However, further work is required to elucidate this mechanism.

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Effect of Condensed Grape Tannins on the *In Vitro* Activity of Digestive Proteases and Activation of Their Zymogens

HOON IL OH and JOHAN E. HOFF

ABSTRACT

Only at concentrations substantially higher than those likely to occur in human diets did grape tannins have a significant adverse effect on the *in vitro* digestion of bovine serum albumin (BSA). The activities of pepsin and chymotrypsin were at such levels (concentrations greater than 0.1%) significantly reduced. In contrast, that of trypsin increased markedly due to denaturation of BSA by the tannins. Tannin concentrations in excess of 0.5% strongly inhibited the activation of chymotrypsinogen, while the activation of trypsinogen by enterokinase was drastically reduced at concentrations as low as 0.05% due to precipitation of the substrate. BSA digestion was markedly reduced in sequential multizymogen experiments at tannin concentrations of C.5% but not at 0.1%.

INTRODUCTION

MANY VEGETABLE and cereal foods, especially fruits, beverages and grain sorghum contain considerable amounts of tannins, mainly of the "condensed tannin" type. These polymeric polyphenolic substances (proanthocyanidins) with molecular weight range of 500–3,000 have the ability to form insoluble complexes with proteins under favorable circumstances (Gustavson, 1956; Calderon et al., 1968; Hagerman and Butler, 1978). Depression of growth of chicks fed high tannin diets (Chang and Fuller, 1964; Vohra et al., 1966; Connor et al., 1969) has been attributed to either reduced protein digestibility, inactivation of digestive enzymes, or reduced feed intake caused by the astringency of tannins, or to a combination of these.

Dietary supplementation with protein reduced the growth depressing effect of tannic acid and sorghum tannins in rats (Lease and Mitchell, 1940; Schaffert et al., 1972). The reduced *in vitro* dry matter disappearance of high tannin sorghum grain was attributed in part to a decreased nitrogen utilization associated with non-digestible protein-tannin complexes. Polyethylene glycol has been used to alleviate the decrease in digestibility of proteins by sorghum tannins (McGinty, 1968). Similarly, Armstrong et al. (1973) found that the addition of 1.0% polyvinylpyrrolidone (PVP) overcame the growth depressing effects.

In vitro studies have shown that various digestive enzymes were significantly inhibited by condensed tannins in the absence of other proteins (Tamir and Alumot, 1969; Milic et al., 1972). However, low concentration of tannins (0.1%, w/v) did not significantly inhibit the activities of various digestive enzymes in the presence of 1% BSA (Oh et al., 1977). Chibber et al. (1978) reported that tannins of sorghum greatly affected *in vitro* digestibility of sorghum proteins via the inhibition of the digestive action of pepsin, trypsin and chymotrypsin.

No information is available in the literature about the effect of tannins on the activation of the zymogens of the major proteolytic enzymes of the gastrointestinal tract. The present study was undertaken to investigate the effects *in vitro* of grape tannins on the activation of these zymogens and on the digestibility of bovine serum albumin (BSA) when the digestive proteases were generated from the zymogens under conditions simulating those of the human gastrointestinal tract.

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MATERIALS & METHODS

Materials

Characterization of the grape tannin preparation used in this investigation has been previously reported (Oh and Hoff, 1979). It consists of a mixture of procyanidins ranging in apparent molecular weight from 900–3000. Enzyme preparations used were as follows: pepsin (EC 3.4.4.1) from hog stomach mucosa, 2x cryst; pepsinogen from hog stomach, cryst.; trypsin (EC 3.4.31.4) from hog pancreas, cryst.; trypsinogen from bovine pancreas, 1x cryst.; α-chymotrypsin (EC 3.4.21.1) from bovine pancreas, 1x cryst.; α-chymotrypsinogen A from bovine pancreas, 6x cryst. and were all purchased from Sigma Chemical Co., St. Louis, MO. Crude enterokinase (EC 3.4.21.9) from pig intestine was obtained from Miles Laboratories. The sources of enzyme substrates for assays were: hemoglobin, Worthington Biochemical Corp.; benzoyl-L-tyrosine ethyl ester (BTEE), p-tosyl-L-arginine methyl ester HCl (TAME), (Sigma Chemical Co.); benzoyl-DL-arginine-p-nitroanilide (BAPNA), Boehringer Mannheim, Germany. BSA (Fraction V) was obtained from Sigma Chemical Co. and PVP from Dickinson and Co.

Models of the digestive systems

Gastric system. The pH and ionic strength simulated those found in the human gastric system (Hollander and Gowgill, 1931; Hollander, 1952). A pH of 1.8, 0.1M ionic strength and 500 μg/mL of pepsin were used.

Intestinal system. The pH of intestinal contents increases from around 6.0 in the duodenum to about 8.0 in the lower ileum (Borgstrom et al., 1957). An average value of 7.0 was chosen. An ionic strength of 0.1M and 500 μg/mL each of trypsin and of chymotrypsin were employed.

Sequential gastric and intestinal system. BSA (10mg mL^{-1}) was first digested for 1 hr by activated pepsin from pepsinogen under gastric conditions. The pH of the reaction mixture was then adjusted to that of intestinal tract (pH 7.0). Trypsinogen, chymotrypsinogen and enterokinase were added, and the mixture further incubated at 37°C for various periods of time. All model mixtures were gently agitated during incubation.

Measurement of digestion

The solution of grape tannins was mixed with the protein solution before addition of enzymes. Reaction mixtures (see legends of figures) were incubated in a water bath for the specified times at 37°C. After incubation the reaction mixtures were treated with 10% trichloroacetic acid (2 mL to 1.4 mL of reaction mixture) and centrifuged for 2 min at 13,000 × *g* in a Brinkman centrifuge (Eppendorf model 3200). The degree of proteolysis in the supernatant was determined with the ninhydrin method according to Spies (1957). Digestion was performed in duplicate. The extent of digestion (% of theoretical maximum) was calculated based on proportion of peptide bonds susceptible to attack by the individual enzymes.

Enzyme assays

Reaction mixtures (see legends of figures) were incubated in a water bath for the specified times at 37°C and centrifuged for 2 min at 13,000 × *g*. All assays were done with the supernatant. Activity of pepsin was determined by the method of Anson (1938) with hemoglobin as the substrate. Tryptic activity was measured by the procedure of Kak-

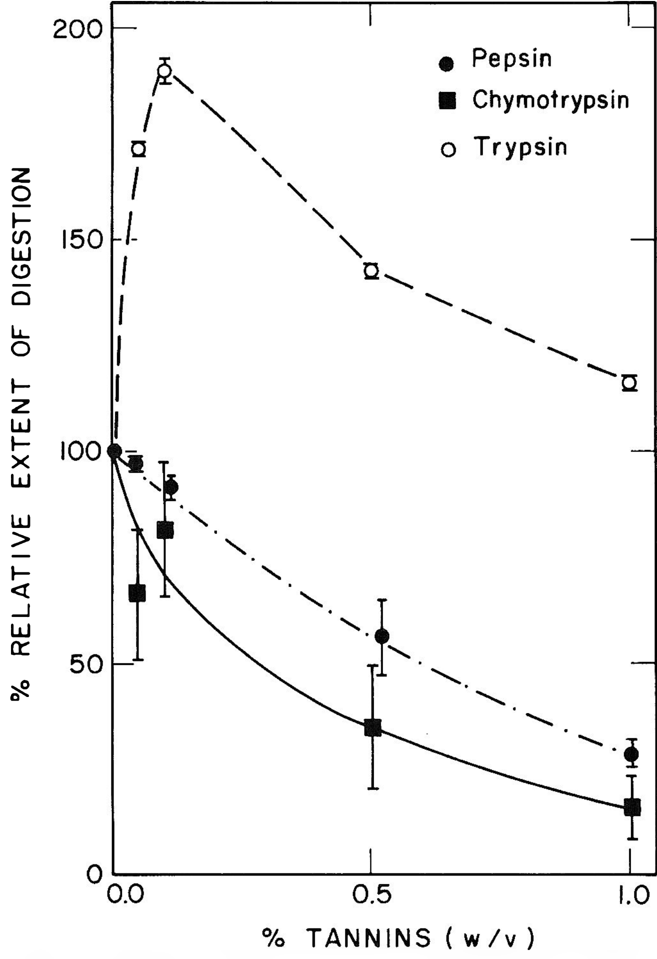

Fig. 1—Effects of tannins on digestion of BSA by pepsin, chymotrypsin, or trypsin under gastrointestinal conditions. Test solution: pepsin 0.8 mL, 0.09% in 0.096N HCl + 0.03M NaCl, pH 1.0; tannin 0.3 mL, 0-4.67% in 0.1M sodium bicarbonate soln., pH 7.0; BSA 0.3 mL, 4.67% in 0.1M sodium bicarbonate soln., pH 7.0. Final pH 1.8. Incubated for 30 min at 37°C. Chymotrypsin or trypsin 0.2 mL, 0.35% in 0.1M MOPS, pH 7.0; tannin 0.2 mL, 0-7% in 0.1M MOPS, pH 7.0; BSA 0.5 mL, 2.8% in 0.1M MOPS, pH 7.0. Mixture made up to 1.4 mL with 0.1M MOPS, pH 7.0. Reaction mixtures for chymotryptic and tryptic digestion were incubated for 20 min and 30 min at 37°C, respectively.

ade et al. (1974) using benzoyl-DL-arginine-p-nitroanilide as the substrate. The chymotryptic activity was determined with benzoyl-L-tyrosine ethyl ester according to Hummel (1959). Enterokinase activity was assayed with trypsinogen using p-tosyl-L-arginine methyl ester HCl as the substrate for trypsin according to the procedure of Baratti et al. (1973).

RESULTS & DISCUSSION

Effect of tannins on digestion of BSA by digestive enzymes

Digestion of BSA by pepsin decreased progressively with increasing tannin concentration. At low concentrations of tannins, digestion of the protein by pepsin was not significantly affected, while it was inhibited by 43% at a tannin concentration of 0.5% and by 72% at a tannin concentration of 1% (Fig. 1). The extent of digestion of the protein by chymotrypsin was affected in a similar manner.

In contrast to digestion by pepsin or chymotrypsin, digestion of BSA by trypsin increased markedly in the presence of tannins up to 0.1% and decreased gradually at higher concentrations (Fig. 1). Even at a tannin concentration of 1%, protein digestion was more extensive than in the absence of tannins.

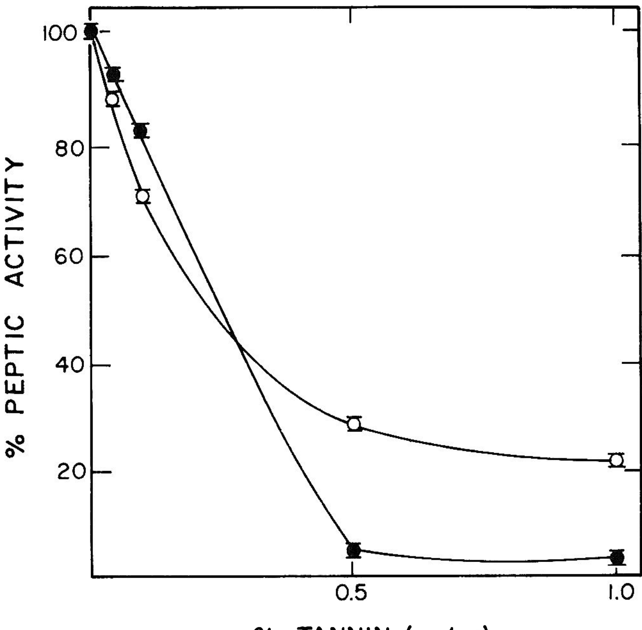

Fig. 2—Effect of tannins on the activation of pepsinogen in absence (o - o) and presence (• - •) of BSA under gastric conditions. Test solution: pepsinogen 0.8 mL, 0.09% in 0.096N HCl + 0.03M NaCl, pH 1.0; tannin 0.3 mL, 0-4.67% in 0.1M sodium carbonate soln., pH 7.0; BSA 0.3 mL, 4.67% in 0.1M sodium bicarbonate soln., pH 7.0. Final pH 1.8. Incubated for 30 min at 37°C.

This may be attributable to deformation of the native BSA structure due to complexing with tannins, thus rendering the protein more susceptible to tryptic attack. Oh et al. (1977) have shown that this positive effect of tannin on digestion disappeared when the protein was denatured prior to digestion. Fully denatured BSA was digested to a greater extent than native protein, and addition of tannin to such denatured protein had a slight negative effect on digestion.

The difference in the effect of tannins on the digestibility of BSA by trypsin and chymotrypsin may be due to their different amino acid specificities. Chymotrypsin has a preference for peptide linkages involving hydrophobic (aromatic) amino acids. It was shown (Oh et al., 1980; Hagerman and Butler, 1980) that the aromatic ring structure of condensed tannins preferentially binds to hydrophobic regions of proteins and poly-α-amino acids. Thus, the hydrophobic amino acids would be the preferred sites for association with tannins and the corresponding peptide linkages therefore rendered resistant to attack by chymotrypsin.

Activation of pepsinogen under gastric conditions of pH and ionic strength simulating those found in a human gastric system showed that a low concentration of tannins (0.1%) slightly inhibited the activation process in the presence of 1% BSA (Fig. 2). Inhibition of activation at high tannin concentrations were more severe in the absence of protein than in its presence and increased with increasing tannin concentration. At tannin concentrations greater than 0.5% (w/v) inhibition of pepsinogen activation was nearly complete. Protein exerted a slight protective role on the inactivation of pepsinogen at low tannin concentrations but failed to show this protective effect at high tannin concentrations.

Activation of chymotrypsinogen by trypsin appeared to increase slightly in the presence of 1% BSA and at low concentrations of tannins (up to 0.1%), while in the absence of the protein, the activation decreased by about 25% (Fig. 3). At tannin concentrations in excess of 0.5%, the activation was drastically inhibited by tannins both in the absence and pres-

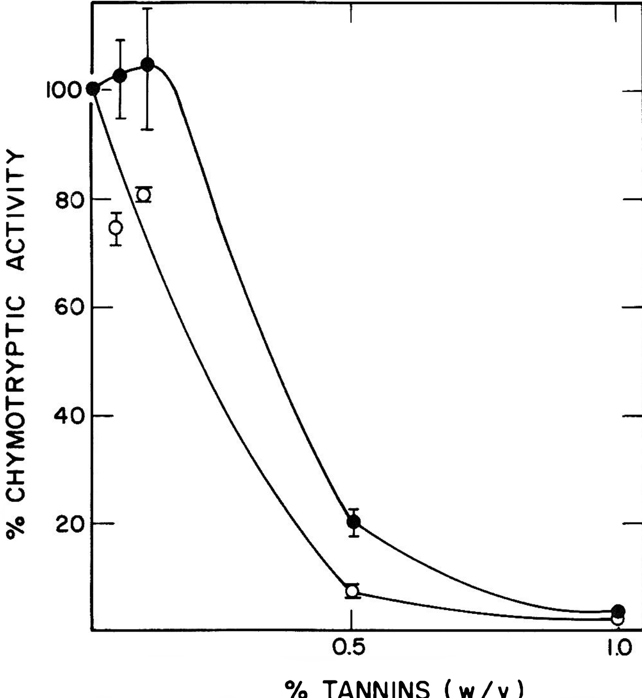

Fig. 3—Effect of tannins on the activation of chymotrypsinogen in absence (○ - ○) and presence (● - ●) of BSA. Test solution: chymotrypsinogen 0.1 mL, 0.2% in 0.001N HCl; trypsin 0.1 mL, 0.01% in 0.001N HCl; tannin 0.1 mL 0-7% in 0.001N HCl; BSA 0.2 mL, 3.5% in 0.15M Tris buffer, pH 7.8. Mixture made up to 0.7mL with 0.15M Tris buffer, pH 7.8. Incubated for 20 min at 37°C.

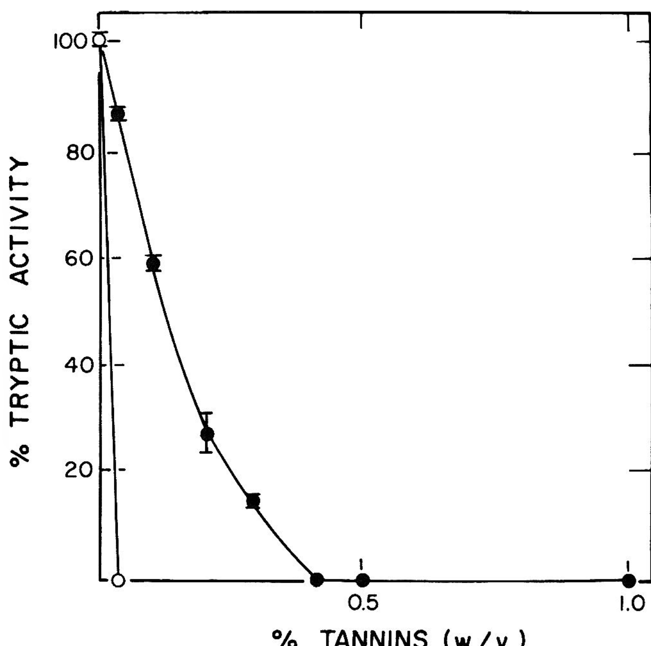

Fig. 4—Effect of tannins on the activation of trypsinogen by enterokinase in absence (○ - ○) and presence (● - ●) of BSA. Test solution: trypsinogen 0.1 mL, 0.1% in 0.001N HCl containing 0.002M $CaCl_2$; enterokinase 0.1 mL, 0.2% in 0.07M sodium succinate buffer, pH 5.6; tannin 0.1 mL, 0-7% in 0.001N HCl; BSA 0.2 mL, 3.5% in 0.001N HCl. Mixture made up to 0.7 mL with 0.07M sodium succinate buffer, pH 5.6. Incubated for 30 min at 37°C.

ence of BSA. When the concentration of tannin was 1.0%, almost no activation of chymotrypsinogen took place. The protective effect of the protein diminished rapidly as the tannin concentration increased.

The effect of tannins on the activation of trypsinogen by enterokinase was studied at pH 5.6 (Maroux et al., 1971). In the absence of BSA, 0.05% tannin completely inhibited the activation process. The inhibition was somewhat alleviated in the presence of the protein, although the effect was still severe (Fig. 4). It was observed that the grape tannins formed a heavy precipitate with trypsinogen, and it is likely that the inhibition was due to substantial removal of the substrate from the reaction mixture rather than to inhibition of the enzyme proper. The potential implications of this finding could be rather important since the activation of trypsinogen to trypsin controls the activation of other pancreatic zymogens such as chymotrypsinogen and procarboxypeptidase which are activated by trypsin. Therefore, it is reasonable to assume that *in vivo* failure to produce sufficient amounts of trypsin might have a severe effect on the proteolytic digestion processes further down the digestive tract. In fact, Haworth et al. (1971) found that patients who are genetically deficient in enterokinase generally suffer from protein malnutrition. Our observations are insufficient to reach any firm conclusions concerning *in vivo* effects. The consequences of substrate precipitation are not necessarily severe since proteolytic enzymes are known to attack insoluble substrates readily. The rate of reaction would furthermore be influenced by the degree of dispersibility of the solids, by their effective surface area, and by the extent of other proteins present. On the other hand, it should be noted that Glick and Joslyn (1970) observed that the intestinal tracts of rats fed high tannin diets contained unusually large amounts of proteolytic enzymes. These could only be detected following addition of enterokinase, indicating that they were originally present in the form of zymogens.

Effect of tannins on digestion of protein by activated pepsin followed by trypsin and chymotrypsin

The effect of tannins on digestiblity of BSA was carried out *in vitro* utilizing the zymogens, pepsinogen, trypsinogen and chymotrypsinogen, simulating the conditions for the human gastrointestinal tract. The protein was first digested for 1 hr under gastric conditions (pH 1.8). The pH was then adjusted to that of the intestinal tract (pH 7), and enterokinase, trypsinogen and chymotrypsinogen added. The mixture was further incubated for various periods of time at 37°C.

Tannins at low concentration (0.1%, w/v) did not significantly decrease the digestion of the protein. However, the extent of protein digestion due to sequential enzymatic actions markedly decreased when tannins were present at the high concentration of 0.5% (Fig. 5). Digestion of the protein by activated pepsin decreased by about 50% at this concentration of tannins, indicating significant interference of tannins with the activation of pepsinogen and/or pepsin activity. While protein digestion in the absence of tannins continuously increased in the intestinal system following peptic digestion, there was virtually no further protein digestion in the presence of high concentrations of tannins. Addition of 0.1% (w/v) PVP partially alleviated the decrease in protein digestion.

The significance of these findings in terms of human nutrition remains to be investigated. These data suggested that the extent to which adverse effects might be experienced would be highly dependent on the amount of tannins ingested. If one assumes that the maximal concentration in the diet of condensed tannins that can be tolerated for sensory reasons is approximately 1% of the food dry matter, and further, that the dry matter has undergone a twentyfold dilution by the time it reaches the duodenum, the tannin concentration would now be 0.05%. At this concentration all the inhibitory effects discussed in this paper, except perhaps enterokinase inhibition, would be insignificant provided adequate amounts of protein were ingested. But concentrations of tannins even substantially

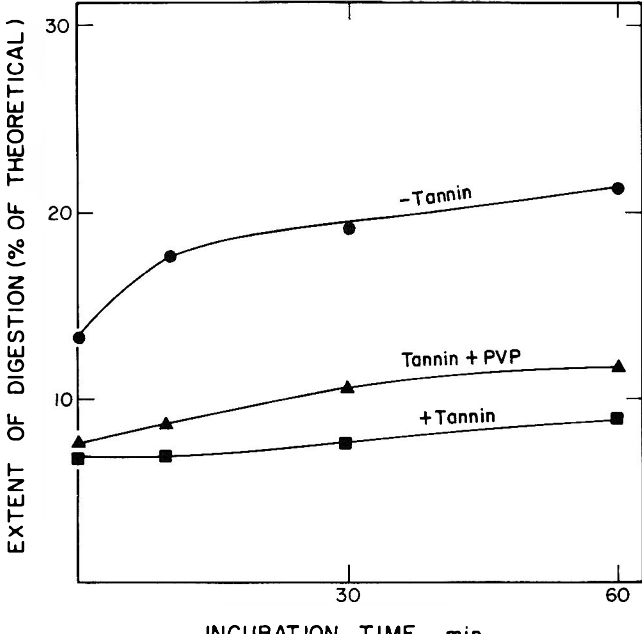

Fig. 5—Effect of tannins (0.5%, w/v) on the digestion of BSA by activated pepsin followed by trypsin and chymotrypsin. Test solution: 5.33% BSA, 0.3 mL with or without 0.53% PVP in 0.1M bicarbonate soln, pH 7.0; tannin 0.3 mL, 2.67% in 0.1M bicarbonate soln, pH 7.0; pepsinogen 0.8 mL, 0.7% in 0.096N HCl + 0.03M NaCl, pH 1.0. After the mixture (pH 1.8) was incubated for 1 hr, 0.1 mL of mixture of chymotrypsinogen (0.8%) and trypsinogen (0.8%) in 0.5M Tris buffer, pH 8.0, and 0.1 mL of enterokinase (0.08%) in 0.5M Tris buffer, pH 8.0 was added and further incubated for various periods of time at 37°C. Final pH 7.0.

higher may be tolerated without substantial adverse effects as suggested by the observations by Mehansho et al. (1983). These investigators found that rats ingesting diets containing up to 7% tannins (sorghum) produced after an adaption period copious amounts of parotid gland high proline proteins with affinities to tannins which are 10-fold greater than that of bovine serum albumin. This suggests that the proteins in saliva may have an important function in tannin detoxification. Our model systems did not incorporate that factor.

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Effects of Heat Treatments on the Ethylene Forming Enzyme System in Papayas

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ABSTRACT

Heat inactivation studies were conducted on the ethylene-forming enzyme system (EFE) in papayas. Heat inactivation of the papaya EFE was biphasic and both phases followed first order kinetics. The activation energies E_a for the thermal inactivation of the heat resistant (HR) and the heat susceptible (HS) EFEs were 68.3 and 51.2 Kcal/mole, respectively. The thermodynamic constants for the heat inactivation of both EFEs were; enthalapy, 51.2 kcal/mole for HS and 66.7 kcal/mole for HR; free energy, 22.5 kcal/mole for HS and 23.3 kcal/mole for HR; entropy, 87.2 cal/deg-mole for HS and 137.8 cal/deg-mole for HR. The heat resistant EFE appeared to compromise about 25% of the total EFE activity. Commercial heat treatments used for quarantine treatments affected mainly the heat susceptible portion which comprised the remaining 75% of the total EFE activity.

INTRODUCTION

PAPAYAS that were expected from Hawaii in the past two decades were usually heat treated by submersion in heated water (49°C) for 20 min to control decay (Akamine and Arisumi, 1953). Recently, a hot water treatment (42°C for 40 min followed by 49°C for 20 min) for the disinfestation of fruit flies was adopted as a quarantine treatment replacing the suspended ethylene dibromide fumigation treatment (Couey and Hayes, 1986). The adoption of the hot water treatment by the papaya industry incurred a few difficulties. Some fruits were inadvertently exposed to excessive temperatures or to prolonged heat treatments resulting in thermally injured fruit which ripened externally with full color development but remained immature internally, retaining a 1- to 1.5-cm-thick area of hard tissue surrounding the seed cavity. The delayed softening of the fruit tissue has been correlated to a decrease in polygalacturonase activity (Chan et al., 1981). Further characterization of papaya polygalacturonases by Chan and Tam (1982) showed these enzymes to be highly heat resistant in the temperature range (42–49°c) used for the quarantine treatments. Hence it was concluded that the deleterious effects of heat were directed to the regulatory mechanisms controlling the generation or activation of polygalacturonase.

Ethylene as a plant hormone has been known to trigger a variety of ripening processes such as the stimulation of polygalacturonase activity in tomatoes (Grierson and Tucker, 1983; Su et al., 1984). 1-Aminocyclopropone-1-carboxylic acid (ACC) has been shown to be the immediate precursor of ethylene (Adams and Yang, 1979). The enzyme converting ACC to ethylene, referred to as the ethylene forming enzyme (EFE), is present in most plant tissues but has yet to be isolated. It is very labile to surfactants, osmotic or cold shocks and heat sensitive. It also is assumed to be membrane bound (Imaseki and Watanabe, 1978; Apelbaum et al., 1981; Yang, 1985; Yu et al., 1980). Therefore, it was postulated that EFE could serve as a simple but effective biochemical indicator of fruit injury due to the heat treatments. In addition, a basic study of the thermodynamics of the heat inactivation of EFE might reveal some characteristics of the EFE system. The objective of this study was to investigate the heat inactivation of the EFE system in papayas.

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MATERIALS & METHODS

Heat inactivation of fruit discs

Heat inactivation studies of plant enzymes are normally performed in vitro on partially or highly purified enzyme extracts in capillary or thin-walled test tubes to minimize problems due to heat penetration. However, attempts to produce an active EFE extract in this laboratory have been unsuccessful; there are no reports in the literature on the successful extraction and isolation of EFE. It appears that intact membranes are requisite for EFE activity (Lurssen et al., 1979; Guy and Kende, 1984). Hence, discs of papaya flesh were used in this study.

Papaya exocarp discs (10-mm diameter × 2.5-mm thick) were removed with a cork borer from 1/2 - 3/4 ripe (50 - 75% yellow) papayas, placed into perforated plastic test tubes (30-mm diameter × 100-mm length), heated in a water bath at prescribed times and temperatures and cooled under running tap water.

Heat inactivation of whole fruits

To determine the effect of heat on the EFE system in papayas the fruit were heat treated according to the procedures prescribed for the quarantine treatments (42°C for 30 min plus 49°C for 20 min) or decay control (49°C for 20 min) as well as for extended times of 40 min at 42°C or 30, 40 and 60 min at 49°C. The fruits were cooled with a water shower (26°C) for 20 min after each heat treatment. Exocarp discs were removed from both the heated and nonheated fruits and then analyzed for EFE activity.

Assay for EFE activity

EFE activity of excised discs was determined by the conversion of administered ACC to ethylene (Wang and Adams, 1982). Ten discs were incubated for 1 hr at 23°C with constant shaking in a sealed 25-mL Erlenmeyer flask containing 15 mL of 1 mM AOAA (aminooxy acetic acid) and 2% KCl, in the presence or absence of 1 mM ACC. Five milliliter of gas were sampled with a syringe and analyzed for ethylene with a Hewlett-Packard Model HP5830 Gas Chromatograph equipped with a flame ionization detector. The carrier gas (helium) flow rate was 31 mL/min. The 1.2 m × 2.3-mm i.d. stainless steel column was packed with Porapak QS 100/120 mesh. The column was operated isothermally at 60°C with the detector temperature at 250°C and the injector at 150°C. EFE activity was expressed as nl of ethylene produced/g fresh weight-hr.

Calculation of thermodynamic constants

The thermodynamic equations as given by Glasstone (1960) and Whitaker (1972) were used to calculate the first order rate constants, k; the energy of activation, E_a; enthalapy, ΔH; free energy, ΔF; and entropy, ΔS. The rate constrants were calculated by iteratively fitting the following equation to the observed data points:

$$\text{EFE activity} = p\, e^{-k't} + (100\text{-}p)e^{-k''t}$$

where p is the percent of the postulated heat resistant enzyme, k′ and k″ are the rate constants of the heat resistant (HREFE) and heat sensitive (HSEFE) ethylene forming enzyme, respectively, and t is the exposure time.

RESULTS & DISCUSSION

THE AVERAGE EFE ACTIVITY of the unheated excised papaya discs was 362 nl/ g-hr which was high in comparison to mung bean hypocotyls, 59 nL/g-hr, and apple plugs, 195

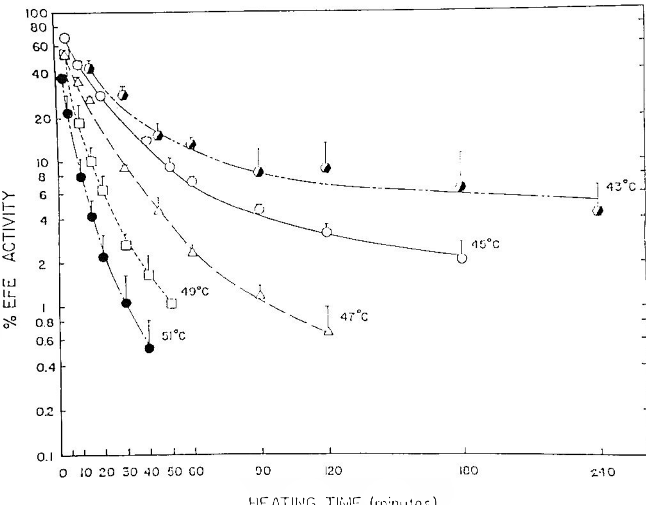

Fig. 1—Heat inactivation of papaya EFE. The means of three replications are shown with ±SD (bar).

Table 1—Kinetic data for heat inactivation of papaya EFE

Temp °C	Rate constant, min^{-1} Heat susceptible	Heat resistant	R^{2b}	D value[a], min Heat susceptible	Heat resistant
42[c]	0.053	0.005	—	42.8	470
43	0.073	0.007	0.996	31.5	311
45	0.102	0.019	0.999	22.5	123
46[c]	0.157	0.021	—	14.7	112
47	0.162	0.034	0.996	14.2	68.5
49	0.242	0.073	1.00	9.52	31.8
51	0.546	0.108	0.999	4.22	21.3
E_a	51.25kcal/mole	68.3kcal/mole			
Q_{10}	12.4	28.6			
ΔH	51.61Kcal/mole	67.67Kcal/mole			
ΔF	22.51Kcal/mole	23.28Kcal/mole			
ΔS	87.23cal/deg-mole	137.8cal/deg-mole			

[a] Decimal reduction times
[b] R^2 = coefficient of determination
[c] Calculated
E_a = energy of activation; Q_{10} = temperature coefficient; ΔH = enthalpy; ΔF = free energy; ΔS = entropy.

nl/g-hr, (Apelbaum et al., 1981). The effects of time and temperature on papaya EFE activity on a semi-logarithmic plot is shown in Fig. 1. If the thermal inactivation of EFE followed first order kinetics, a straight line would have been obtained. Inspection of Fig. 1 indicates a composite thermal inactivation curve which could be resolved graphically into two straight lines as shown in Fig. 2. This indicated the presence of two types of EFE of which one type was more heat resistant than the other. The existence of biphasic first order curves in other enzyme systems have generally suggested either the presence of isozymes as in the cases of papaya invertase (Chan and Kwok, 1976), papaya catalase (Chan et al., 1978), papaya acid phosphatase (Carreno and Chan, 1982) and corn peroxidase (Yamamoto et al., 1962) or the presence of multiple forms of enzymes as in the case of papaya exo- and endo-polygalacturonases (Chan and Tam, 1982). In the case of the EFE system, the interpretation of the presence of biphasic thermal inactivation curves must be restricted at this time to only the existence of at least two forms of EFEs with different heat resistances. This restriction was necessitated because the EFE system has not been isolated and characterized. The rate constants (k) and the D values for the two forms of EFE are listed in Table 1. The coefficients of determination, r^2, ranged from 0.9959 to 0.9999 (Table 1). The stabilities of the enzymes are presented as D Values or decimal reduction times which are defined as the time required for 90% destruction of enzyme activity at constant temperature (Yamamoto et al., 1962). As shown in Table 1 the D values for HREFFE are considerably larger than the D values for HSEFE indicating that much longer heating times are required for the heat inactivation of HREFE.

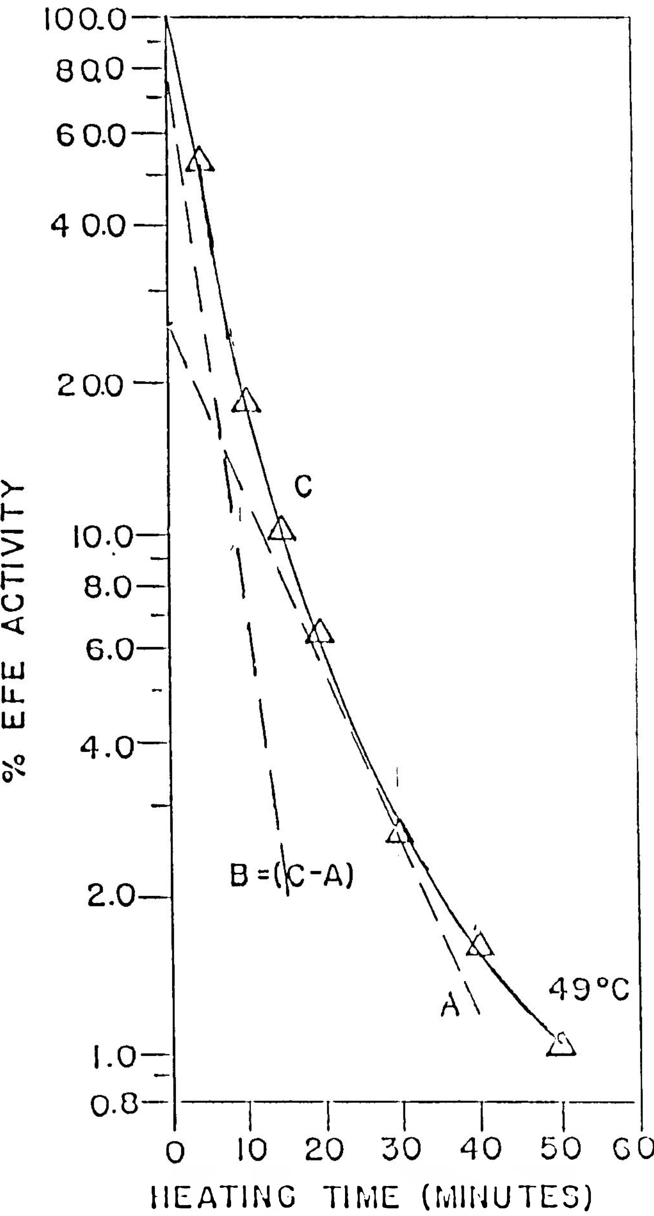

Fig. 2—Graphic resolution of the composite heat inactivation curve at 49°C for papaya. The observed heat inactivation curve (C) when subtracted from the heat resistant EFE (A) results in the heat sensitive EFE activity (B).

An extropolation of the thermal inactivation curve for the HREFE at 49°C to zero heating time indicates that the heat resistant portion comprises about 25% of the total EFE activity with the heat susceptible portion accounting for the remaining 75% (Fig. 2). It is most likely that the quarantine heat treatments (42°C for 30 min plus 49°C for 20 min) affected mainly the heat susceptible portion of the papaya EFE system. This was due to the fact that during the course of the quarantine heat treatments the internal temperatures at a depth of 2.5 mm of whole papayas attained only 47.8°c due to the asymptotic conduction of heat through the fruit (Couey and Hayes, 1986). Only during the final 12 min of the heat treatment were temperatures greater than 46°C attained within 2.5 mm of the fruits surface. At 46-47°C the D values for the HREFE (Table 1) indicate very little inactivation at these temperatures. In contrast HSEFE would suffer considerable losses in activity as judged by its D values at similar temperatures.

The amount of EFE activity remaining in whole papayas

Table 2—Effects of extended commercial heat treatments on papaya EFE activity[a]

Heating time, min	% Remaining total EFE activity			
	Immersed in H_2O @ 49°C for x min		Immersed in H_2O @ 42°C for x min + 49°C for 20 min	
		SD		SD
20	29.6	8.7	—	—
30	18.2	12.7	14.1	7.3
40	8.9	4.7	12.4	6.5
60	2.3	1.0	—	

[a] Each heat treatment was replicated three times using whole fruits.

that were heat treated for 20, 30, 40, and 60 min at 49°C is shown in Table 2. The EFE activity in discs removed after heat treatment of whole fruits at 49°C (Table 2) is considerably greater than the EFE activity remaining in discs that were excised and then heated at 49°C (Fig. 1). The differences can be attributed to the less severe heat treatment experienced by the discs from heat treated whole fruit due to slower conduction of heat through the fruit. The thermal inactivation of EFE in whole fruits heated at 49°C appeared to be equivalent to a heat treatment of excised discs at 46°C (Fig. 1).

The amount of EFE activity in discs removed from whole fruits that had been subjected to the quarantine heat treatments of either 30 or 40 min at 42°C followed by 20 min at 49°C is listed in Table 2. The shorter time of 30 min was recently adopted as an approved quarantine treatment in an effort to alleviate the incidences of thermally injured fruits, however, as shown in Table 2 the differences in EFE activity were less than 2% but more interesting is the considerable loss in EFE activity due to the quarantine heat treatments (12-14%) compared to the losses caused by the decay control heat treatment (29.6%).

The activation energies (E_a) for thermal inactivation of HREFE and HSEFE were 68.3 and 51.2 kcal/mol, respectively. The higher E_a for the inactivation of the HREFE was significant, because, according to the Arrhenius equation small changes in temperature will cause large changes in the reaction-rate constant, k. Hence the Q_{10} for the HREFE (28.6) was 2.31 times larger than the Q_{10} for the HSEFE (12.4). Such a large Q_{10} for the HREFE indicated that a heating temperature that was a few degrees in excess of the prescribed heat treatments would cause a significant increase in the rate of heat inactivation. This fact is especially important to packing house operators where the specified heat treatment of 49°C for 20 min results in 23.5% residual HREFE activity whereas 50°C and 51°C for the same 20 min heating period will result in 21.1 and 11.5% HREFE activity remaining. In contrast errors in excess of the specified heat treatment of 20 min by 1, 2 and 3 min will result in 21.8, 20.3 and 18.9% HREFE activity. Hence, in regards to the quarantine treatment, excessive temperatures were more injurious to the EFE system than were excessive heating times. HSEFE of papaya appeared to be one of its most heat labile enzyme systems. Its E_a compared to the E_a of some of its other enzymes was smaller; endo-polygalacturonase (92 Kcal) and exopolygalacturonase (102 Kcal) (Chan and Tam, 1982); catalase I (85.9 Kcal) and catalase II (97.0 Kcal) (Chan et al., 1978).

The free energies, ΔF, for both HREFE (23.3 Kcal/mole) and HSEFE (22.5Kcal/mole) were in the range (20–30 Kcal/mole) considered by Eyring and Stearn (1939) to be characteristic for thermal denaturation (Table 1). The entropy, ΔS, for the HREFE was 137.8 cal/deg-mole and for the HSEFE, 87.2 cal/deg-mole. Such large positive ΔSs suggested that the inactivation of both HREFE and HSEFE was accompanied by an unfolding of either the enzymes' polypeptide chain or its membrane matrix into a less highly ordered, more random structure. The higher ΔS for the HREFE compared to the ΔS for the HSEFE also implied that the HREFE undergoes a greater change in structure in its transition state than the HSEFE.

The number of noncovalent bonds broken on denaturation of a protein can be estimated if an average ΔH of 5000 cal/mole is used per bond (Whitaker, 1972). By dividing each EFE's enthalpy, ΔH, by 5 Kcal/mole the number of non-covalent bonds broken in HSEFE and HREFE were 10 and 13 bonds, respectively. Hence the HREFE was most likely in a more ordered state because it had more noncovalent bonds stabilizing its native structure; therefore, its high entropy change was due to a greater loss of order commencing from a more ordered state than the HSEFE and resulting in a similar same state of disorder, i.e., a random coil.

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Preparation and Evaluation of Supercritical Carbon Dioxide Defatted Soybean Flakes

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ABSTRACT

Full-fat soybean flakes were extracted with supercritical carbon dioxide ($SC-CO_2$) at pressures of 10,600–12,400 psi, temperatures from 80–100°C, and moisture levels of 5–13.5%. Conditions could be selected to produce defatted soybean meals with nitrogen solubility indices greater than 70% and flavor scores greater than 6.5 on a scale of 1 to 10 (1 = strong and 10 = bland). The usual grassy-beany and bitter flavors of hexane-defatted soybean flours were only minimally detectable in the optimally $SC-CO_2$-extracted materials. Bland, defatted soybean meal prepared by $SC-CO_2$ extraction was further processed into high-quality protein concentrates and isolates that were stable when stored under adverse conditions.

INTRODUCTION

THIRTY MILLION TONS of soybeans are extracted each year in the United States for domestic use. Virutally all these soybeans are extracted with hexane or, perhaps, hexane-alcohol mixtures. Hexane extraction of soybeans produces defatted protein products that generally have grassy-beany and bitter tastes associated with them (Kalbrenner et al., 1971), which renders them undesirable for food use. Investigators have suggested that these flavors are not only breakdown products of lipid oxidation (Sessa et al., 1969), but may also be caused by traces of hexane residues in the meal products (Warner et al., 1983). Liquid CO_2 has been used to remove volatile substances such as aroma constituents from fruits and other food products (Schultz et al., 1974).

Supercritical carbon dioxide ($SC-CO_2$) has many advantages over hexane extraction because it is nontoxic, nonexplosive, cheap, readily available, and easily removed from extracted products. $SC-CO_2$ is also a more versatile solvent than liquid CO_2 because extraction conditions can be varied over a wider range.

The $SC-CO_2$ extraction of oilseeds, including soybeans, has been reported (Friedrich et al., 1982), but analytical and flavor studies of $SC-CO_2$-extracted soybean meals are needed to evaluate the potential of these defatted products.

The purpose of this study is to determine the optimum extraction parameters for $SC-CO_2$ extraction of full-fat soybean flakes to produce defatted protein products with improved flavor characteristics and high protein solubility.

MATERIALS & METHODS

Materials

Commercial-grade carbon dioxide was obtained in 60-lb cylinders from Matheson (Joliet, IL). Certified seed grade TS-280 variety soybeans, 1980 crop year, were purchased from Sommer Brothers Seed Company, Pekin, IL. Soybeans were cracked, dehulled, and flaked to produce full-fat flakes 0.01 inch thick. Sufficient quantities of flakes were either dried in a vacuum oven at 30°C or humidified at 30°C in closed vessels to produce flakes with known moisture levels. After the full-fat flakes were prepared, they were stored at −20°C.

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Extraction equipment and conditions

The general apparatus for $SC-CO_2$ extractions in a laboratory has been discussed by Friedrich et al. (1982), Friedrich and List (1982), and Friedrich and Pryde (1984) and is shown in Fig. 1. In this study, we required accurate temperature control. To accomplish this, a small 316 SS extractor (22" × 0.56" i.d.) with a pressure rating of 20,000 psig was placed in a gas chromatography oven and controlled within + ½°C of desired extraction temperature.

Experimental design

To optimize the extraction parameters, a central composite rotatable design was used to produce response surface contours (Cochran and Cox, 1957). Three conditions (pressure, temperature and moisture) were varied while CO_2 flow and extraction time were constant. To establish the time parameter, an extraction was run at the lowest combination of pressure and temperature (84°C and 11,000 psi) and a flow rate of 15 standard L of CO_2 per min. The time required to reach a residual oil content of <1% in the meal was determined to be 20 min. At combinations of higher pressure and temperature the time required for extraction was less, but the extraction was continued for the full 20 min to eliminate the time variable. The pressure was varied from 10,600 to 12,400 psi, temperature from 80 to 100°C, and moisture from 5 to 12.4%. In addition to the moisture in the flakes, 1 mL of water was added to a glass wool plug at the inlet of the extractor for each of the extractions. This was necessary because in the absence of additional water the dry CO_2 entering the column carries the moisture away from the initially contacted flakes before the flavor-improving effects of moisture, pressure and temperature are realized. After 20 extractions conditions were defined, their order was randomized, and the extractions were conducted.

Full-fat flakes (40g) with known moisture levels were removed from cold storage and placed in the tube extractor. The extractor was sealed and brought to desired pressure while being heated to a controlled temperature. With a CO_2 supply pressure of 1,100–1,200 psi, a flow of 15–18 standard L/min was maintained at extraction conditions. After 20 min the extractor was depressurized, and the defatted flakes were removed.

To verify the time, flow, moisture, temperature, and pressure relationships, several larger confirmatory extractions (i.e., 1 kg) at selected conditions were conducted. Parameters for the extractions were selected based on the data in Fig. 2. $SC-CO_2$ flows were adjusted (85–95 Std L/min) to allow complete extraction in 20 min.

Sensory evaluation

A 15-member trained panel, experienced in tasting soybean protein products, evaluated the samples for flavor quality. The samples were prepared as 2% dispersions in 25°C carbon-filtered tap water. Panelists were served 10 mL aliquots of each dispersion in 50 mL glass beakers covered with watch glasses. All sensory testing was conducted under red fluorescent light to mask color differences between samples. Further details on testing procedures were described previously by Warner et al. (1983). Overall scores were based on a 10-point scale, with 10 as bland (excellent quality) and 1 as strong (poor quality). Individual flavor descriptions (cereal, cooked bean, grassy, bitter, astringent and toasted) of the samples were rated on a scale of 0 = none, 1 = weak intensity, 2 = moderate intensity, and 3 = strong intensity. Flavor intensity values (FIV's) were calculated by a procedure described by Warner et al. (1983). A wheat flour control (2% dispersion) was included in all tests.

A balanced incomplete block design (Cochran and Cox, 1957) was used as a testing pattern for the 20 samples. The samples were randomly divided into two groups of 10 samples each. Each tester evaluated three samples at a panel session. A total of nine scores for each sample was used in calculating overall means. An analysis of variance

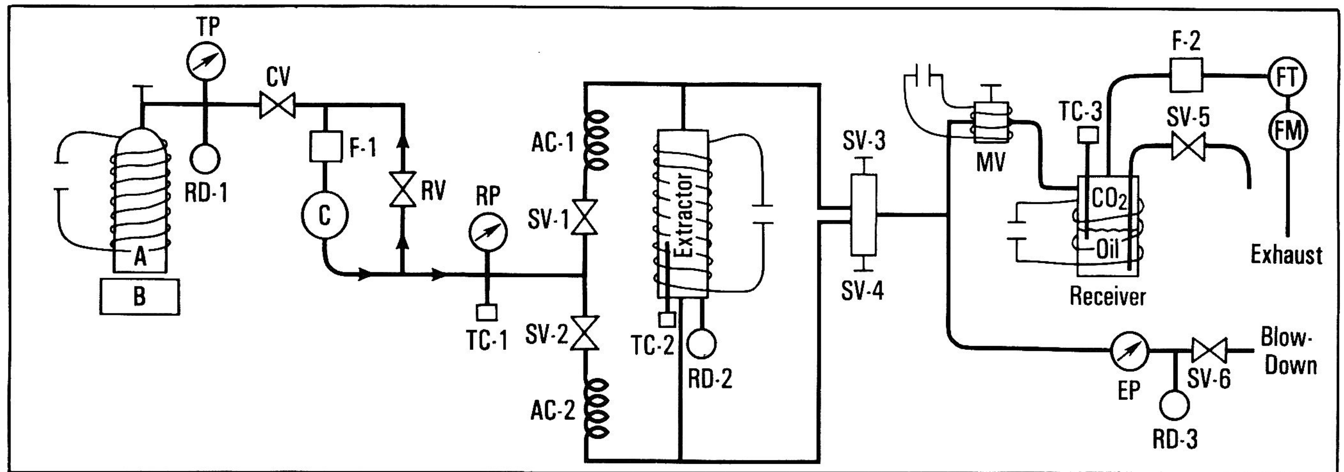

Fig. 1—Supercritical carbon dioxide extraction apparatus: (A) CO_2 cylinder, (B) balance, (TP) tank pressure, (RD1-3) rupture disc assemblies, (CV) check valve, (F1&2) gas filters, (C) diaphragm compressor, (RV) back pressure regulating valve, (RP) regulated gas pressure, (TC1-3) thermocouples, (SV1-6) shut-off valves, (AC1&2) gas coolers, (MV) micrometering valve, (FM) flow meter, (FT) flow totalizer, and (EP) extractor pressure.

Table 1—Effect of supercritical carbon dioxide extraction conditions on NSI, flavor score, and enzyme activity

	Extraction conditions[a]						
Sample	PSI	°C	H_2O, %	NSI[b]	FS[c]	LU[d]	TI[e]
1	11,500	100	9.0	48	6.9	0	17.5
2	11,500	90	5.0	82	4.8	589	31.3
3	11,500	90	9.0	67	6.3	3	21.6
4	11,500	90	9.0	68	6.6	0	19.4
5	11,500	90	9.0	57	6.7	8	19.9
6	11,500	90	9.0	65	7.2	0	27.3
7	11,500	90	9.0	69	6.2	0	27.5
8	11,500	90	9.0	69	6.0	ND	27.7
9	11,500	90	12.4	62	6.4	16	29.5
10	11,500	80	9.0	80	5.8	779	31.3
11	11,000	84	6.5	80	4.2	688	ND
12	11,000	84	11.4	63	6.1	57	27.3
13	11,000	96	6.5	67	6.8	0	22.8
14	11,000	96	11.4	33	7.2	0	10.0
15	12,000	84	6.5	81	5.6	625	21.4
16	12,000	84	11.4	62	7.2	3	24.8
17	12,000	96	6.5	72	6.0	0	28.2
18	12,000	96	11.4	34	7.3	0	ND
19	10,600	90	9.0	66	6.3	10	27.7
20	12,400	90	9.0	69	6.6	80	27.4

[a] In all extractions, time and carbon dioxide flow remained constant.
[b] Nitrogen solubility index (NSI); a measure of protein solubility.
[c] Flavor score; 1 to 10 where 1 is strong and 10 is bland.
[d] Lipoxygenase units; µM O_2 consumed/min/mg protein.
[e] Soybean trypsin-inhibitor activity, mg/g.
ND = not determined.

determined statistical significance among means at the 95% confidence level (P>0.05). Concentrates and isolates were evaluated in groups of two or three and data was analyzed statistically by a two-way analysis of variance (P>0.05). Least significant difference (LSD) among samples was also calculated (P>0.05).

Analytical methods

The following American Oil Chemists' Society Methods (1975) were used in this study: oil, Ba 3-38; moisture, Bc 2-49; protein, Ba 4-38; residual oil, Ac 3-44; nitrogen solubility index, Ba 11-65; and urease, Ba 9-58. Lipoxygenase (EC 1.13.1.13) was determined by the method described by Christopher et al. (1972). Soybean trypsin-inhibitor (TI) activity of the defatted meals was determined by the method of Hamerstrand et al. (1981).

RESULTS & DISCUSSION

Extraction of flakes

The extraction conditions, nitrogen solubility index (NSI), flavor scores (FS's), and lipoxygenase activity of 20 samples are shown in Table 1. Where a high NSI and FS are desired, the best extraction conditions were at least 12,000 psi, about 85°C, and moisture levels of 10.5 to 11.5%. Such conditions should give defatted soybean flakes with NSI's approaching 70 and FS's near 7.0.

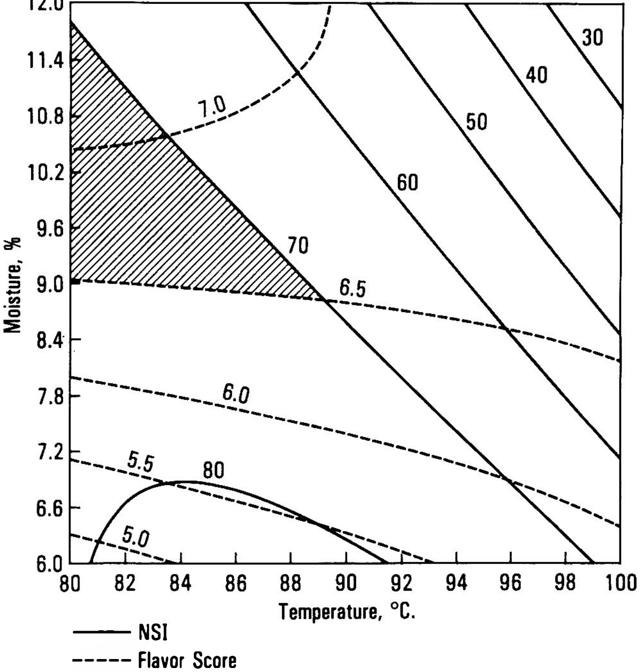

Fig. 2—Contour diagram showing effect of moisture and temperature on nitrogen solubility index (——) and flavor score (----) when full-fat soybean flakes are extracted at 12,000 psi for 20 min with constant carbon dioxide flow.

Samples with high NSI's but very low FS's had lipoxygenase activities very similar to undenatured hexane-defatted soybean flakes. Apparently the presence of moisture in the flakes caused denaturation of lipoxygenase, with a concurrent increase in flavor score but a decrease in NSI (see samples 11 and 12, Table 1, for example). However, one sample (sample 17) had a high NSI and a low flavor score but had no lipoxygenase activity. This result is unexplainable at this time.

Full urease activity was indicated in all 20 samples by a consistent pH change of 2.0–2.1. The reasons why lipoxygenase was denatured and urease was unaffected are of interest

Table 2—Equations relating NSI and FS to process condition[a]

$$NSI = -863.31 + 6.62058P + 10.79693T + 33.28007M - 0.03715P^2 - 0.07172T^2 - 0.17133M^2 + 0.02875PT - 0.05407PM - 0.31689TM$$

$R^b = 0.962^{**}$

$$FS = -132.13 + 0.84087P + 1.71314T + 1.56402M + 0.00125P^2 - 0.00006T - 0.04444M^2 - 0.01296PT + 0.00753PM - 0.01549TM$$

$R = 0.934^{**}$

[a] P = pressure/100, T = temperature, M = % moisture.
[b] R = multiple correlation. Correlation of observed and predicted NSI or FS values.
** Significant at Probability >0.01 level.

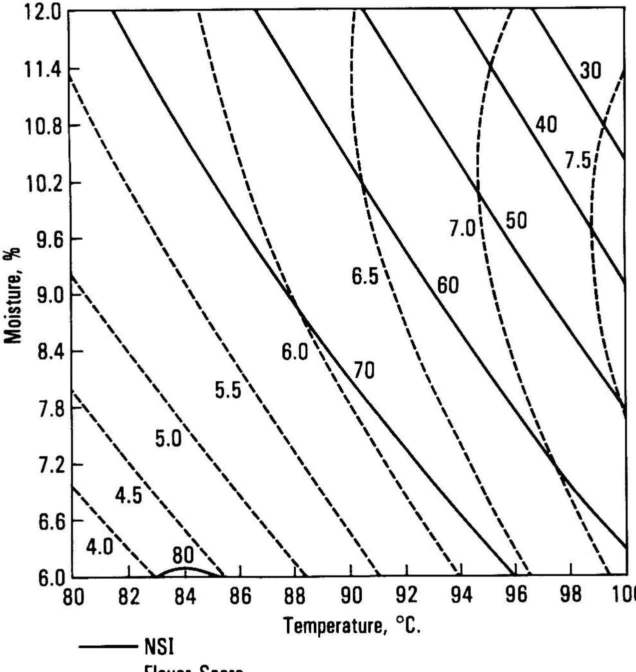

Fig. 3—Contour diagram showing effect of moisture and temperature on nitrogen solubility index (——) and flavor score (---) when full-fat soybean flakes are extracted at 11,000 psi for 20 min with constant carbon dioxide flow.

and currently under investigation. Weder (1980, 1984) reported that ribonuclease and lysozyme were changed slightly when treated with wet SC-CO_2 at ~5000 psi at 80°C. The changes were mainly in the disulfide bonds of the proteins, and this may be the phenomenon we have observed.

The NSI and FS data in Table 1 were analyzed statistically, and equations were derived that predicted either NSI or FS when pressure (P), temperature (T), and moisture (M) were controlled for a given time and CO_2 flow rate (Table 2). These equations were used to create NSI and FS contours when temperature and moisture were varied. Figure 2 is the plot for 12,000 psi and Fig. 3 is for 11,000 psi. Products with NSI's greater than 70 and FS's greater than 6.5 can be obtained by extracting under conditions designated by crosshatching in Fig. 2. Products with these qualities were unattainable when the pressure of the extraction was only 11,000 psi (Fig. 3). When the pressure of the extraction was decreased, the NSI's generally remained the same, but there was a significant decrease (probability >0.05) in FS's. Table 3 shows the NSI and flavor scores of two 1 kg batches of defatted soybean flour. The NSI of sample 1 was lower than anticipated (~68); however, the FS were as predicted (~7) by Fig. 2. The second defatted soy sample had the expected NSI (~47) and flavor score (~7). The FS of soy flour #1 (7.3) was rated as not significantly different than a wheat flour control (FS = 7.9) whereas the FS of sample #2 (6.6) was significantly lower than the control.

There are three procedures for preparing soybean protein concentrates (Kalbrener et al., 1971) which contain 70% protein. The above two defatted soybean flours were used to prepare concentrates by (a) water leaching of toasted defatted soybean flakes, (b) alcohol washing of undenatured defatted soybean flakes, or (c) acid leaching of defatted soybean flakes. Flavor scores ranged from 6.1 for the acid-leach concentrate to 7.2 for the alcohol wash concentrate (Table 3). The scores for concentrates prepared by acid or water leaching were rated significantly lower than the wheat flour control (FS = 7.9). There were no significant differences between the score of the control and those of the two alcohol-washed concentrates. The acid-leached concentrate had a predominant grassy flavor and the water-leached sample was described as having cereal and toasted flavors, whereas both the wheat flour control and the alcohol-washed concentrate were only described as cereal-like. All samples had low FIV's for bitter and astringent characteristics.

Soybean protein isolates, i.e., 90% protein, were also prepared from the same two flours by procedures described by Kalbrener et al. (1971). The flavor evaluation of these two isolates is also shown in Table 3. Flavor scores were 7.0 and 6.1, respectively, for the samples extracted at 12,000 psi. Both isolates were rated as significantly lower in score than the wheat flour control (FS = 7.9) possibly because of the bitter taste in both isolates and the astringency in isolate #2. Neither of the isolates had any undesirable grassy flavors.

The flours, concentrates, and isolate (Table 3) were also evaluated for flavor after storage at 37°C for 2 months. No significant differences were observed between the flavor scores of the aged flours, concentrates or isolates and their corresponding control samples held at 2°C. The intensities of predominant flavors in the samples also did not change significantly after sample aging for 2 mo.

Flavor scores of the flours, concentrates and isolates listed in Table 3 compared favorably with scores of commercial soy products published by Warner et al. (1983). The authors reported flavor scores of commercial soy flours ranging from 5.5 to 6.3 whereas scores for optimally processed SC-CO_2 samples in this study were 7.3 and 6.6. The concentrates and isolates (Table 3) also received higher scores than the top-rated corresponding commercial products. Flavor intensity of descriptions such as cereal and toasted were low in SC-CO_2 processed samples because the heat treatment steps typically required for commercially prepared soy products were not necessary.

To examine the storage stability further, we prepared a partially defatted soybean flour (2.8% residual oil) by SC-CO_2 and compared the flavor of a portion of the sample stored at 2°C for 2 months against a portion stored at 37°C for the same time. No significant difference was noted in the flavor scores (6.9 and 6.7, respectively). The FIV's on the sample were also nearly identical. This observation, and the fact that flours prepared by SC-CO_2 extraction had less lipoxygenase activity than hexane-extracted flakes would suggest that the enzyme inactivation may protect the defatted soybean meal from developing undesirable flavors during storage.

In conclusion, it has been observed that full-fat soybean flakes can be extracted with supercritical carbon dioxide at pressures of 10,600-12,500 psi at various temperatures and moistures. By carefully controlling parameters, products with high protein solubility and excellent flavor scores and profiles can be produced. Flours, concentrates and isolates prepared from SC-CO_2-extracted flakes were also stable after 2 months storage tests at 37°C. The process should have potential in the food industry for the extraction of vegetable oils to yield proteins of superior flavor and physical qualities. The high NSI opens opportunities for high protein beverages and the bland

Table 3—Flavor scores and Flavor Intensity Values (FIV) of fresh and stored soybean products prepared by supercritical carbon dioxide extraction

Samples	Flavor scores[b]	Flavor intensity values[a]					
		Cereal	Cooked bean	Grassy	Bitter	Astringent	Toasted
1 Soy flour[c]	7.3 (7.1)[d]	0.6 (0.6)	0.3 (0.0)	0.5 (0.7)	0.5 (0.2)	0.4 (0.3)	0.0 (0.0)
2 Soy flour[e]	6.6 (6.7)	0.9 (0.5)	0.3 (0.0)	0.4 (0.5)	0.6 (0.4)	0.0 (0.4)	0.0 (0.0)
3 Heat concentrate[f]	6.8 (6.7)	0.6 (1.0)	0.0 (0.4)	0.4 (0.3)	0.3 (0.0)	0.3 (0.3)	0.6 (0.7)
4 Heat concentrate[g]	6.8	1.3	0.0	0.0	0.3	0.3	0.6
5 Alcohol concentrate[f]	7.2 (7.8)	0.4 (0.7)	0.0 (0.0)	0.4 (0.0)	0.5 (0.3)	0.3 (0.2)	0.0 (0.0)
6 Alcohol concentrate[g]	7.1	0.9	0.0	0.2	0.5	0.3	0.0
7 Acid concentrate[f]	6.1 (6.5)	0.3 (0.7)	0.0 (0.3)	0.8 (0.5)	0.8 (0.5)	0.4 (0.6)	0.0 (0.0)
8 Acid concentrate[g]	6.2	0.3	0.0	0.9	0.4	0.8	0.0
9 Isolate[f]	7.0 (6.1)	0.4 (0.6)	0.0 (0.0)	0.0 (0.6)	0.7 (0.6)	0.0 (0.5)	0.0 (0.0)
10 Isolate[g]	6.1	0.7	0.0	0.0	0.6	0.7	0.0

[a] Flavor Intensity Value (FIV) 1 = weak; 2 = moderate; 3 = strong.
[b] Flavor Scores (FS) 1 = strong; 10 = bland; Least significant difference (LSD) = 0.7 ($P>0.05$).
[c] 12,000 psi; 82°C; 11.3% moisture, NSI = 58.
[d] Values in ( ) are for samples stored at 37°C for 2 months.
[e] 12,000 psi; 90°C; 13.5% moisture, NSI = 53.
[f] From flour sample 1.
[g] From flour sample 2.

flavor should encourage the use of these storage stable products in enriched flours for soups, baked goods, etc.

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Protein-Protein Interactions Between Soybean Beta-conglycinin (B_1–B_6) and Myosin

I. C. PENG and S.S. NIELSEN

ABSTRACT

Protein-protein interactions between soybean beta-conglycinin (B_1–B_6) and myosin were studied by turbidity, solubility and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Turbidity and solubility studies showed that, under the experimental conditions used, these proteins interacted at temperatures between 60° and 100°C, while SDS-PAGE analysis indicated that the interaction also occurred at 50°C. The interaction was such that no detectable complexing between these two proteins was observed. The presence of beta-conglycinin resulted in diminished aggregations of myosin heavy chains between 50° and 100°C.

INTRODUCTION

PROTEIN-PROTEIN interactions are important in the gelation phenomenon of food proteins. Food protein gels are the result of protein molecules interacting to form an ordered three dimensional network. Water, fat droplets, salts and other smaller particles are trapped within the network (Schmidt, 1981). Thus, the strength (firmness) of the gel is dependent on the strength of the interacting forces acting on the protein network.

A heat-set gel system such as frankfurters containing high levels of soy protein (>20%) has poor cohesiveness and soft texture (Sofos et al., 1977; Comer, 1979). This is undoubtedly due to poor protein-protein interactions among the proteins in the system.

Beta-conglycinin, which belongs to the 7S protein fraction, is a major soybean protein fraction comprising 25–30% of the total protein. It is composed of several isomeric forms (B_o–B_6) containing three subunits (α, α', and β) in different ratios (Thanh and Shibasaki, 1976a,b; Yamauchi et al., 1981). In contrast, another major soy protein, glycinin or 11S globulin, is a single protein which consists of both acidic and basic polypeptide components (Peng et al., 1984; Nielsen, 1984).

Interactions between glycinin and the major functional muscle protein, myosin, have been investigated (Yamamoto et al., 1973; Peng et al., 1982a,b; Haga et al., 1984). Glycinin interacts with myosin only at temperatures of 85°C and higher. Basic polypeptide components of glycinin are the ones interacting with myosin heavy chains, while the acidic polypeptide components are not involved.

The objective of this research was to use a model system simulating the chemical environment that exists in pasteurized processed meat systems to investigate the interactions between soybean beta-conglycinin (B_1–B_6) and skeletal muscle myosin.

MATERIALS & METHODS

Buffer solution

Purified beta-conglycinin and myosin suspended in a 58.3 mM sodium phosphate buffer (32.5mM Na_2HPO_4, 25.8 mM NaH_2PO_4, 0.4M NaCl, 0.02% NaN_3) were used throughout the experiments. This buffer was designed to provide a chemical environment similar to that which exists in pasteurized processed meat systems (μ, 0.53; pH, 6.5). The buffer composition has been reported previously (Peng et al., 1982a) with the exception that ATP and 2-mercaptoethanol were not included in this study.

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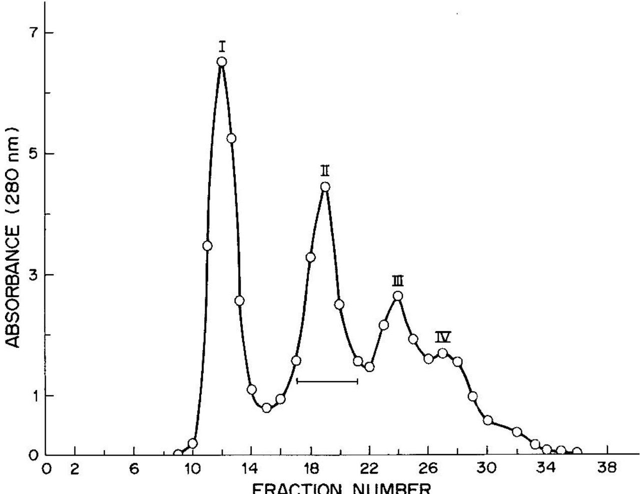

Fig. 1—Elution profile of beta-conglycinin prepared by the method of Thanh and Shibasaki (1976a) from a Sepharose 6B gel filtration column. Bar under peak II indicates fractions pooled for further purification.

Preparation of soy beta-conglycinin (B_1–B_6)

Commercially available defatted soy flour was used for the preparation of beta-conglycinin. The beta-conglycinin (B_1–B_6) was first separated from the glycinin fraction by the Tris-HCl extraction procedure of Thanh and Shibasaki (1976a). B_o-conglycinin usually appears as a contaminant in the glycinin fraction by this procedure (Yamauchi et al., 1981), and was not included in the soy protein preparations used in this study. Beta-conglycinin (B_1–B_6) was further purified by gel filtration (Sepharose 6B column) and affinity chromatography (ConA Sepharose 4B) (Coates et al., 1985). Tris-HCl beta-conglycinin resolved into 4 peaks on the Sepharose 6B column (Fig. 1). Peak II was further purified on the ConA Sepharose 4B column. Beta-conglycinin was adsorbed to the ConA column and was eluted with a 0.1M α-methyl-D-mannoside solution. The purified protein was stored in sodium phosphate buffer at 4°C for less than 1½ months. Protein degradation during storage was minimal as judged by SDS-polyacrylamide gel electrophoresis.

Preparation of myosin

Myosin was prepared from chicken breast muscle (Pectoralis major) by the salting-out method of Quass and Briskey (1968). Myosin, in sodium phosphate buffer, was stored at 4°C and used for the experiments within 1 wk of preparation.

Protein determination

Protein concentrations were derived according to the procedure of Bradford (1976). Bovine serum albumin was used as the standard.

Turbidity test

Myosin and beta-conglycinin solutions individually (2 mg/mL) or in combination (2 mg/mL of each protein) were heated in sodium phosphate buffer at various temperatures (30–100°C) for 30 min and cooled in an ice bath immediately after heating. Turbidity curves were

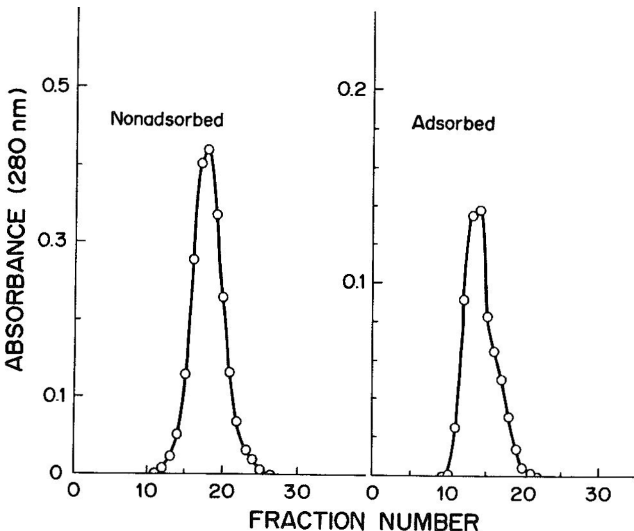

Fig. 2—Elution profiles of the nonadsorbed and adsorbed fractions of the pooled peak II (Fig. 1) further purified on a Con A Sepharose 4B affinity chromatography column. The adsorbed fraction was used as the purified beta-conglycinin.

established shortly after cooling by reading the absorbance (660 nm) at room temperature.

Solubility test

The protein solutions treated above were centrifuged at 50,000 × g for 30 min at 4°C. Protein concentrations of the supernatants and the pellets were determined. Solubility was defined as:

$$\%\ \text{Solubility} = \frac{\text{Protein in supernatant (mg)}}{\text{Sum of protein in supernatant (mg) and pellet (mg)}} \times 100\%$$

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the procedure of Laemmli (1970). The acrylamide concentration of the separating gel was 7.5% (pH 8.9) and that for the stacking gel was 3.5% (pH 6.4).

The results reported here are representative of the experiments replicated three times.

RESULTS & DISCUSSION

The gel filtration elution profile of beta-conglycinin isolated by the Thanh and Shibasaki procedure are shown in Fig. 1. Peak I was very turbid (milky) and contained no detectable protein. Peaks II, III, and IV all contained beta-conglycinin subunits (α, α′ and β) with varying degrees of contamination from glycinin (SDS-PAGE data not shown). Peak II was used for further purification on a ConA-Sepharose 4B column because the purity of Peak II was much greater than that of Peaks III and IV.

The elution profiles of the nonadsorbed and adsorbed portions of Peak II on the ConA-Sepharose 4B affinity column are shown in Fig. 2. Adsorbed protein was used without further purification.

Turbidity test

The effect of heating on the turbidity of myosin, beta-conglycinin and a mixture of the two proteins is shown in Fig. 3. Beta-conglycinin solutions remained relatively clear throughout the temperature range 30–100°C. This minimal increase in turbidity as a function of temperature at ionic strength of 0.5 has been observed by others (Hashizume and Watanabe, 1979). The turbidity of chicken myosin shows two maxima at 60° and 90°C. The second increase in turbidity around 90°C was not observed with rabbit myosin (Peng et al., 1982a), which may indicate that chicken myosin generally behaves differently than

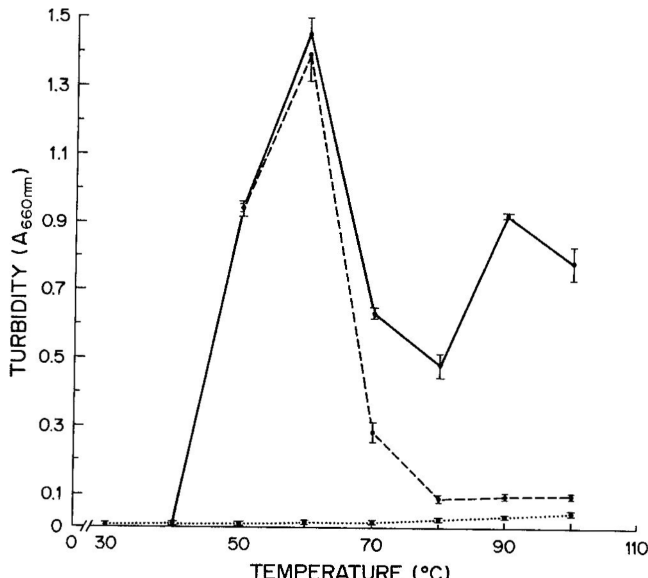

Fig. 3—Turbidity curves of protein solutions upon heating. Bars denote S.E. ···· Beta-conglycinin, —— Myosin, ---- Mixture of beta-conglycinin and myosin.

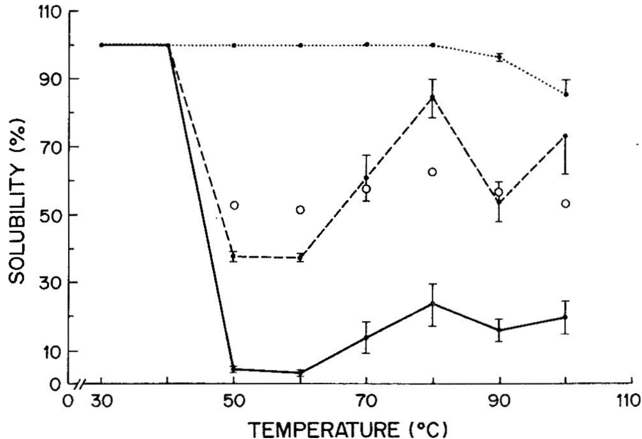

Fig. 4—Solubility curves of protein solutions upon heating. Bars denote S.E. ···· Beta-conglycinin, —— Myosin, ---- Mixture of beta-conglycinin and myosin.

rabbit myosin. The position of the turbidity curve for the protein mixture indicated that an interaction occurred between these two proteins at temperatures of 60–100°C.

Solubility test

The effect of temperature on the solubility of these proteins is shown in Fig. 4. The solubility of beta-conglycinin remained high throughout the temperature range 30–100°C. Chicken myosin showed two minima in solubility, at 60° and 90°C, respectively, complementing the turbidity data. Solubility of the protein mixture also had two minima at 60° and 90°C. The theoretical solubility values of the protein mixture if no interaction takes place (open circles, Fig. 4) are the means of the separate values for myosin and beta-conglycinin. Between 50° and 100°C, the position of the solubility curve of the mixture generally deviated from these theoretical values. Thus, solubility data implied that interactions occurred between myosin and beta-conglycinin upon heating at 50–100°C.

The turbidity and solubility data mentioned above clearly showed that an interaction occurred between myosin and beta-conglycinin when these two proteins were heated at temperatures between 50° (or 60°) and 100°C.

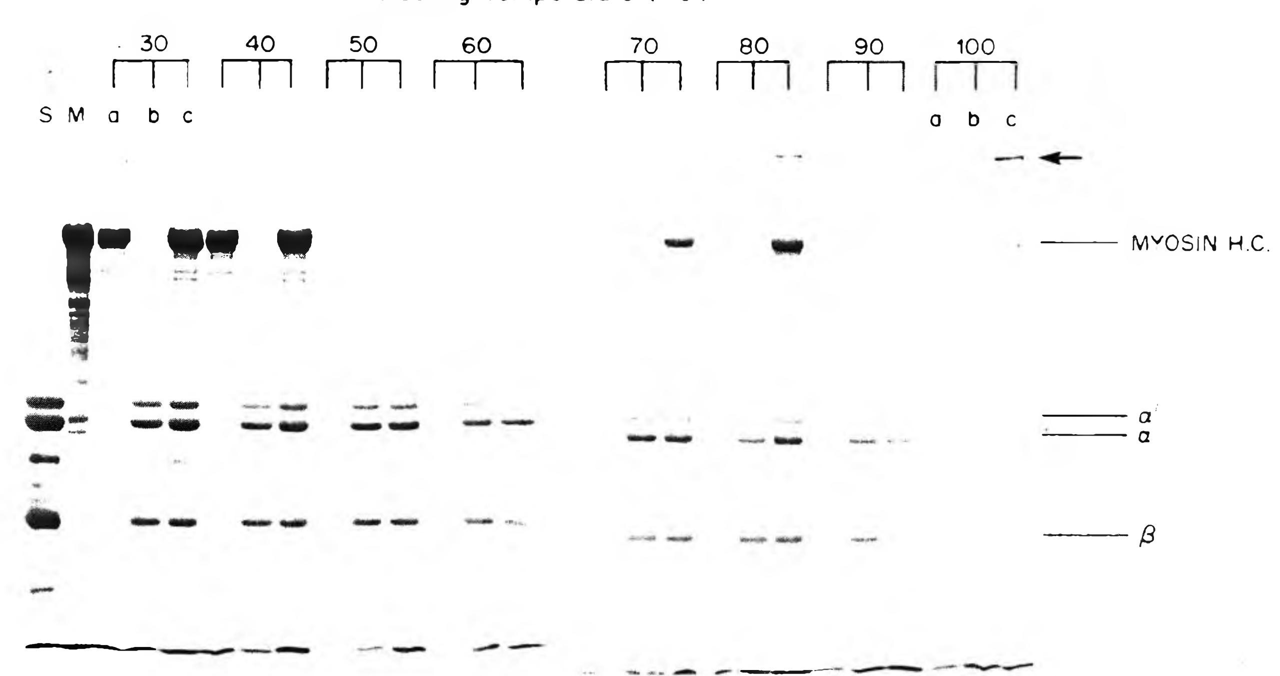

Fig 5—SDS-PAGE band patterns of the supernatants of protein solutions heated at temperatures from 30–100°C. The arrow indicates position of the contaminating salt-soluble protein. S = purified beta-conglycinin (50 μg) M = purified myosin (50 μg). a,b, and c = supernatants of myosin, beta-conglycinin, and mixture of these two proteins, respectively. α, α' and β = subunits of beta-conglycinin.

SDS-polyacrylamide gel electrophoresis

The interaction between the proteins was further investigated with SDS-polyacrylamide gel electrophoresis. The proteins were heated singly and in combination. Equal volumes of supernatants were loaded onto a slab gel to ensure that the intensities of protein bands were proportional to the concentrations of proteins in the supernatants. At 30° and 40°C, no detectable protein interaction occurred since the intensities of the proteins in the mixture were similar to those for individual protein components (Fig. 5). At 50–100°C, the intensities of the beta-conglycinin protein bands in the mixture were the same as for those of the beta-conglycinin heated alone. However, the myosin heavy chain (H.C.) bands in the mixture (Track C) were more intense than those of myosin heated in the absence of beta-conglycinin. The presence of beta-conglycinin seemed to interfere with the self aggregation of myosin heavy chains. This resulted in fewer myosin aggregates in the pellets upon centrifugation and increased amounts of myosin H.C. in the supernatants.

In contrast to our results, King (1977) observed a complex formation between myosin and soybean 7S globulin at temperatures of 75–100°C. His study and ours differ in three ways: In his study, (1) beta-conglycinin was prepared by a different method (Roberts and Briggs, 1965); (2) the myosin used in his study was prepared from rabbit muscle, which apparently behaves differently from chicken myosin as mentioned previously; and (3) a higher pH (7.6) was used.

Thermal denaturation of beta-conglycinin (B_1–B_6) is a function of ionic strength. At ionic strengths of 0.1–2.0, aggregation of dissociated subunits occurs upon heating (Iwabuchi and Shibasaki, 1981). The heat-induced dissociation products of beta-conglycinin are not completely unfolded and they retain their secondary and tertiary structures (Iwabuchi and Yamauchi, 1984). Additionally, the surface hydrophobicity of beta-conglycinin may actually decrease upon heating as evidenced by one tryptophan residue being transferred from the surface to the hydrophobic interior of the molecule (Yamagishi et al., 1982).

Aggregation and precipitation of myosin H.C. was observed when myosin solution was heated (Fig. 3 and 5). This thermal aggregation presumably involved hydrophobic interactions between myosin heavy chains since hydrophobic interactions are enhanced at higher temperatures (Nemethy et al., 1963) and because the buffer used had a high salt concentration.

Thus, the observed interference with aggregations of myosin heavy chains by the presence of beta-conglycinin at 50–100°C (Fig. 3 and 5) may be due to a decreased surface hydrophobicity of beta-conglycinin. Therefore, a high concentration of beta-conglycinin may act as a diluent for the presumed hydrophobic interactions among myosin heavy chains, which results in diminished aggregations of myosin heavy chains.

The myosin preparation used in this study was contaminated by a very slight amount of salt-soluble protein. The subunits of this protein were too large to move into the separating gel and appeared at the top of the separating gel (Fig. 5, Track M). The identity of this contaminating protein is not known, although it could be nebulin or titin which have high molecular weights (Robson, 1983). The presence of beta-conglycinin apparently also interfered with the aggregation of the contaminating protein, since there was an increased intensity of the corresponding protein band (on top of the separating gel) in the mixture, especially from 70–100°C.

In general, frankfurters made with chicken meat have a rubbery texture as compared to those made with beef. The diminished aggregations of myosin heavy chains in the presence of beta-conglycinin may be used to reduce the rubbery texture of the chicken frankfurters.

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Antinutritional Factors in Moth Bean (*Vigna aconitifolia*): Varietal Differences and Effects of Methods of Domestic Processing and Cooking

SANTOSH KHOKHAR and B. M. CHAUHAN

ABSTRACT

Significant varietal differences were observed in the contents of phytic acid, saponin and trypsin inhibitor activity of four varieties of Moth bean (*Vigna aconitifolia* Jacq.). Tannins and lectins were absent. The dry seeds were given different treatments including soaking, sprouting and cooking and the changes in the level of the antinutritional factors were estimated. Soaking the seeds in plain water and mineral salt solution for 12 hr decreased phytic acid to the maximum (46–50%) whereas sprouting for 60 hr had the most pronounced saponin lowering effect (44–66%). The other methods of processing were less effective in reducing the levels of these antinutritional factors. The processing methods involving heat treatment almost eliminated trypsin inhibitor activity while soaking and germination partly removed the activity.

INTRODUCTION

GRAIN LEGUMES are rich and less expensive sources of dietary proteins and contribute substantially to the protein content of the diets of a large part of the world's population. Moth bean (*Vigna aconitifolia* Jacq.) being one of the most drought resistant pulses is widely grown under rainfed conditions in semi-arid and arid zones of India. Spreading growth habits of Moth bean plant, useful in checking soil erosion, provide an additional advantage of growing this legume in these areas.

Presence of antinutritional factors is one of the main drawbacks limiting the nutritional and food qualities of the legumes (Liener, 1976). Phytate, widely distributed in food grains (de Boland et al., 1975), lowers the bioavailability of minerals (Davies and Nightingale, 1975; Erdman, 1979) and inhibits several proteolytic enzymes and amylases (Singh and Krikorian, 1982; Deshpande and Cheryan, 1984). Saponins, present as triterpene glycosides in significant amounts in legume grains (Oakenfull, 1981), impart bitter taste to these plant foods and cause physiological disturbances and toxicity to human system (George, 1965). Besides, saponins have been reported to assert a physiological effect on lowering the level of plasma cholesterol (Oakenfull et al., 1979; Cheeke, 1976). All legumes, studied to date, have been found to contain trypsin inhibitors in varying amounts (Liener, 1976). Trypsin inhibitors when ingested by man in significant amounts disrupt the digestive process and may lead to undesirable physiological reactions (Booth et al., 1960). Genetic manoevring implied in evolving new high yielding varieties may produce wide variation in the contents of these antinutritional factors. For effective utilisation of grain legumes for human nutrition removal/elimination of these undesirable attributes is necessary.

Grain legumes in India are processed and consumed in a variety of forms. The most common method of preparation is usually to soak them overnight and then to cook until they are soft. Other traditional methods include pressure cooking, sprouting, cooking of sprouted seeds, parching and fermentation. Rockland et al. (1979) have developed quick-cooking beans based on soaking the beans in a solution of mineral salts.

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Shortening of cooking time by this method is important as it involves convenience and saving of valuable fuels. It is important to know the extent to which the antinutritional factors survive these domestic processing and cooking treatments and finally remain in the food form in which it is eaten. The objective of this study was to investigate the antinutritional factors in four varieties of Moth bean and the changes brought about on soaking the grains in plain water and in mineral salt solution, ordinary and pressure cooking of presoaked seeds, sprouting and cooking of sprouted seeds.

MATERIALS & METHODS

Materials

The seeds of four high yielding varieties of Moth bean (*Vigna aconitifolia* Jacq.) namely Badami, Jadia, Jawala and Karni were obtained from the Moth Bean Breeder, S.K. N. College of Agriculture, Jobner (Rajasthan) India.

Processing and cooking methods

The methods of processing and cooking included soaking in plain water, soaking in mineral salt solution, ordinary cooking of soaked seeds, pressure cooking of seeds soaked in plain water and mineral salt solution, sprouting and ordinary cooking of sprouts.

Soaking. Seeds were first cleaned and freed from broken seeds, dust and other foreign materials and then soaked either in tap water mixed salt solution (2.5% sodium chloride, 1% sodium tripolyphosphate, 1.5% sodium bicarbonate and 0.5% sodium carbonate, all w/v, in distilled water) for 12 hr at 37°C. A seed to water ratio of 1:5 (w/v) was used. The unimbibed water was discarded. The soaked seeds were rinsed twice in distilled water and then dried in hot air oven at 55°C.

Ordinary cooking. Presoaked seeds after rinsing in distilled water were put in round mouth tall beakers fitted with condensers. After adding tap water (three times the weight of dry seeds), the samples were cooked on a hot plate until they became soft when felt between fingers. Cooked seeds along with cooking water were dried at 55°C.

Pressure cooking. Presoaked seeds were cooked for 20 min at 1.05 kg/cm^2 pressure in a pressure cooker. Dry seeds to cooking water ratio of 1:2 (w/v) was used. The cooked sample was mashed and then dried at 55°C.

Sprouting. The soaked seeds were germinated in sterile petri-dishes lined with wet filter papers for 60 hr at 25°C, with frequent watering. The sprouts were rinsed in distilled water and dried at 55°C. The rinsed sprouts were then cooked till soft like soaked samples mentioned above and then mashed and dried at 55°C. The oven dried unprocessed as well as processed samples were milled to pass through 0.5 mm sieve and stored in plastic containers until required for further analysis.

Chemical analysis

Phytic acid was extracted in 0.5M nitric acid and determined by the method of Davies and Reid (1979). The method of Gestetner et al. (1966) was employed for extraction and colorimetric determination of saponins. Trypsin inhibitor activity (TIA), both from processed and unprocessed Moth beans, was extracted in 0.05M phosphate buffer (pH 7.0). TIA was determined by incubation of inhibitor extracts at 37°C for 20 min at pH 7.6 (phosphate buffer 0.1M) using casein as substrate (Roy and Rao, 1971). Proteins were estimated by the method of Lowry et al. (1951). Trypsin activity was expressed in terms of trypsin units. One unit of trypsin was defined as the amount of trypsin which converted one mg casein to TCA soluble components at 37°C

Table 1—Level of antinutritional factors in Moth bean—varietal differences[a]

Variety	Phytic acid (mg/100g)	Saponin (mg/100g)	Trypsin inhibitor activity (units/10g)	Tannins	Lectins
Badami	888 ± 9	3349 ± 18	2719 ± 139	Traces	Absent
Jadia	866 ± 8	3073 ± 14	2031 ± 111	Traces	Absent
Jawala	852 ± 7	2953 ± 12	1676 ± 89	Traces	Absent
Karni	899 ± 8	2833 ± 18	1897 ± 149	Traces	Absent
CD (5%)	21	35	376		

[a] Values, the means of six independent determinations, are expressed on dry matter basis.

for 20 min at pH 7.6. One unit of TIA was that which reduced the activity of trypsin by one unit under the assay conditions. Tannins were extracted by refluxing sample in methanol for 4 hr and determined colorimetrically with HCl-vanillin reagent (Burn, 1971). Catechin was used as a reference standard. All samples were extracted within 6 hr of milling. Haemagglutinating activity was determined by the method of Liener (1955). The data were statistically analysed by standard method of analysis of variance (Snedecor and Cochran, 1967).

RESULTS & DISCUSSION

Phytic acid

Phytic acid among Moth bean cultivars ranged from 852 to 899 mg/100g (Table 1). Karni had the highest amount followed in descending order by Badami, Jadia and Jawala. The level of phytic acid in Moth bean cultivars seemed to be lower than that reported for blackgram (Reddy et al., 1978), kidney beans (Lolas and Markakis, 1975), and soybeans (de Boland et al., 1975), thus suggesting that nutritive value of raw Moth bean seeds would be impaired to a comparatively lesser extent. Besides lowering the bioavailability of minerals (Davies and Nightingale, 1975) and inhibiting the digestibility of proteins (Singh and Krikorian, 1982) and carbohydrates (Deshpande and Cheryan, 1984), phytate is also reported to lead to hard-cook in pulses (Boulter, 1982). The last undesirable attribute increases cooking time of legume grains and concerns people in developing countries where energy sources including fuel wood are becoming increasingly scarce and expensive.

Soaking was the first step, and all other processing and cooking methods followed it. Upon comparing the absolute loss of phytic acid during different processing methods (Table 2), it was observed that soaking of seeds in plain water or mixed salt solution was the most effective method of lowering phytic acid of Moth bean grains. Pressure cooking of presoaked seeds in mixed salt solution and sprouting for 60 hr decreased phytate by 4–7% and 2–6%, respectively. Other treatments had only negligible diminishing effect. Apparent cumulative loss of phytic acid appeared to be the highest during cooking of sprouted seeds and pressure cooking of seeds presoaked in mixed salt solution. But, since the seeds were first soaked and then processed for sprouting and pressure cooking, the major contribution for the observed loss was due to soaking only. The obvious decrease in phytate of Moth bean during soaking can be attributed to leaching of phytate ions into soaking water under the influence of concentration gradient. Such losses may be taken as a function of changed permeability of seed coat. Absorption of water in seeds may also activate phytase resulting in hydrolysis and hence loss of phytic acid.

Comparatively less loss of phytic acid during germination than that reported for pulses in earlier studies (Reddy et al., 1978; Tabekhia and Luh, 1980; Ologhobo and Fetuga, 1984) may be due to the fact that in this study the seeds were germinated at a relatively lower temperature than the optimum required (45–50°C) for phytase activity. Iyer et al. (1980) observed a reduction in the phytate concentration of dry beans during the combined process of soaking and cooking. Cooking the beans in water containing 3% NaCl at 100°C for 3 hr resulted in a slight decrease in phytic acid in kidney beans, mung and pink beans (Tabekhia and Luh, 1980).

Soaking, an integral part of traditional methods of processing legume grains in India, thus offers the dual advantage of saving energy costs by shortening cooking time as well as rendering the grains nutritionally superior by removing certain antinutritional factors like phytic acid.

Saponin

Moth bean was found to be a fairly rich source of saponin and the concentration among cultivars varied significantly from 2833 to 3349 mg/100g on dry matter basis. Similar significant differences in saponin of different cultivars have been observed in navy beans (Fenwick and Oakenfull, 1983). Moth bean had relatively less saponin content than that reported for chickpea and soybeans (Fenwick and Oakenfull, 1983). The results are consistent with those reported (Oakenfull, 1981) indicating that legumes are one of the most significant sources of saponins in man's diet.

Saponin contents of the beans were greatly diminished by sprouting (56–66%) (Table 3). Soaking in mixed mineral salt solution also removed considerable amounts (30–36%) but soaking in plain water decreased saponin content only slightly (9–18%). Pressure cooking of seeds presoaked in tap water reduced saponin levels more effectively than when seeds were presoaked in mineral salt solution. This difference may be due to the fact that the soaking in mixed salt solution had already removed a great amount of saponin and a relatively smaller amount was left when the seeds were pressure cooked. Ordinary cooking of the seeds presoaked in plain water reduced saponin level by 12–15% but ordinary cooking of sprouted seeds decreased saponin only marginally (2–6%). The loss of saponin from seeds during soaking may perhaps be attributed to the leaching of saponin into the soaking medium through simple diffusion. Since mixed salt solution is known to alter the permeability of seed coat, comparatively greater loss of saponins from Moth beans during soaking in mineral salt solution may be attributed to enhanced seed coat permeability. Loss during cooking may indicate the thermolabile nature of saponins. Whether cooking results in the formation of poorly extractable complex between saponins and sugars or amino acids is not known. Enzymatic degradation, which may be a possible explanation of saponin loss during germination, is also not established.

The results here contradict the earlier report (Fenwick and Oakenfull, 1983) that saponins survive the rigors of cooking and food processing but support the view that processing like fermentation results in more than 50% loss of saponins (Potter et al., 1979; Stakacs and Madas, 1979).

Trypsin inhibitor activity

Antitryptic activity in the Moth bean cultivars varied significantly (Table 1). Badami had the highest activity followed in descending order by Jadia, Karni and Jawala. When compared with other common legume seeds like navy beans, chickpea, lentil and black gram as reported earlier (Gallardo et al., 1974), the trypsin inhibitor activity (TIA) in Moth bean was considerably low. Subbulakshmi et al. (1976) also found that horse gram contained twelve times more trypsin inhibitor activity than that of Moth bean. But Meshram et al. (1980) reported that Moth bean was free from TIA. This suggested that TIA in Moth bean was under genetic control.

Mixed salt solution removed TIA of Moth beans to a greater extent than plain water (Table 4). Since trypsin inhibitors are low molecular proteins, their extraction from the seed to the

Table 2—Effect of domestic processing and cooking methods on the level of phytic acid in Moth bean[a]

Treatment	Variety			
	Badami	Jadia	Jawala	Karni
Soaking in plain water	477 ± 2 (46)	434 ± 3 (50)	448 ± 7 (49)	474 ± 1 (47)
Ordinary cooking of pre-soaked seeds	468 ± 1 (1)	414 ± 2 (2)	442 ± 2 (1)	460 ± 1 (2)
Pressure cooking of pre-soaked seeds	449 ± 5 (3)	408 ± 3 (3)	415 ± 2 (4)	467 ± 2 (1)
Soaking in mineral salt solution	442 ± 3 (50)	431 ± 2 (50)	450 ± 2 (47)	451 ± 2 (50)
Pressure cooking of seeds presoaked in mineral salt solution	382 ± 3 (7)	394 ± 2 (5)	408 ± 3 (5)	411 ± 2 (4)
Sprouting	456 ± 2 (2)	386 ± 2 (5)	423 ± 2 (3)	426 ± 1 (6)
Cooking of sprouted seeds	446 ± 5 (2)	364 ± 2 (3)	418 ± 3 (1)	419 ± 2 (0)
	CD (5%) = 5			

[a] Values are averages of six independent determinations expressed on dry matter basis as mg/100g. Figures in parentheses represent loss of phytate, exclusively during the treatment, as percentage of control values.

Table 3—Effect of domestic processing and cooking methods on the saponin contents of Moth bean[a]

Treatment	Variety			
	Badami	Jadia	Jawala	Karni
Soaking in plain water	2932 ± 68 (13)	2814 ± 49 (9)	2511 ± 61 (15)	2322 ± 68 (18)
Ordinary cooking of presoaked seeds	2500 ± 92 (12)	2330 ± 63 (15)	2122 ± 37 (13)	1899 ± 68 (15)
Pressure cooking of presoaked seeds	1754 ± 40 (35)	1688 ± 47 (36)	1212 ± 32 (44)	1138 ± 48 (42)
Soaking in mineral salt solution	2212 ± 60 (34)	2153 ± 49 (30)	1902 ± 30 (36)	1884 ± 45 (34)
Pressure cooking of seeds presoaked in mineral salt solution	1906 ± 43 (9)	1712 ± 22 (14)	1412 ± 51 (16)	1448 ± 18 (15)
Sprouting	976 ± 14 (58)	758 ± 30 (66)	721 ± 13 (61)	726 ± 36 (56)
Cooking of sprouted seeds	774 ± 10 (6)	691 ± 30 (3)	650 ± 27 (2)	607 ± 24 (5)
	CD (5%) = 57			

[a] Values are averages of six independent determinations expressed on dry matter basis as mg/100g. Figures in parentheses represent loss of saponin, exclusively during the treatment, as percentage of control values.

Table 4—Effect of domestic processing and cooking methods on trypsin inhibitor activity of Moth bean[a]

Treatment	Variety			
	Badami	Jadia	Jawala	Karni
Soaking in plain water	2447 ± 125 (10)	1930 ± 105 (5)	1525 ± 78 (9)	1859 ± 96 (2)
Ordinary cooking of presoaked seeds	293 ± 74 (79)	155 ± 19 (87)	112 ± 19 (84)	171 ± 20 (89)
Pressure cooking of presoaked seeds	63 ± 0 (88)	63 ± 0 (92)	63 ± 0 (87)	63 ± 0 (95)
Soaking in mineral salt solution	1767 ± 91 (35)	1462 ± 25 (28)	1160 ± 37 (31)	1369 ± 22 (28)
Pressure cooking of seeds presoaked in mineral salt solution	63 ± 0 (63)	63 ± 0 (69)	63 ± 0 (65)	63 ± 0 (69)
Sprouting	1141 ± 58 (48)	1001 ± 60 (46)	821 ± 4 (42)	1138 ± 70 (38)
Cooking of sprouted seeds	63 ± 0 (40)	63 ± 0 (46)	63 ± 0 (45)	63 ± 0 (57)
	CD (5%) = 111			

[a] Values are averages of six independent determinations expressed on dry matter basis as units/10g. Figures in parentheses represent loss of trypsin inhibitor activity, exclusively during the treatment, as percentage of control values.

soaking medium is quite possible. The sodium salts present in the soaking medium are likely to increase the porosity of seed coat. This is, perhaps, one of the reasons for greater loss of TIA from the seed in the mixed mineral salt solution. Deshpande and Cheryan (1983) reported about 1 and 13% losses of TIA when dry beans were soaked in distilled water and mixed mineral salt solution, respectively. Ordinary cooking, pressure cooking after soaking in plain water or salt solution and ordinary cooking of sprouts almost eliminated the TIA from the Moth bean seeds. This may be due to the well established heat labile nature of trypsin inhibitors. Cooking and autoclaving have been reported to be effective in inactivating protease inhibitors in several food legumes (Manorama and Sarojini, 1982; Tan and Wong, 1982; Ologhobo and Fetuga, 1983; Gupta and Wagle, 1980).

Germination lowered considerably but could not completely remove TIA from Moth bean seeds. The reductions may be attributed to the mobilization and enzymatic degradation of

proteins including trypsin inhibitors of seeds during germination. Subbulakshmi et al. (1976) reported 37% loss in TIA on germination of Moth bean seeds.

Tannins and lectins

Tannins in the Moth bean seeds were not present in quantitative amounts (Table 1). Tannins are characteristically present in the legume grains having pigmented seed coat and decortication results in removal of 83–97% of tannins. The seed coat of the Moth bean cultivars was almost colorless which may explain the negligible amount of tannins present in them. White colored varieties of dry beans were not found to contain detectable amounts of tannins (Deshpande and Cheryan, 1983). On account of lack of tannins, the Moth bean seeds are anticipated to have better nutritive value than other pulses having significant levels of tannins which are known to affect digestibility of protein, carbohydrates and fat, and bioavailability of minerals (Prabhavathi, 1979; Griffiths and Moseley, 1980). Lectins were found to be absent in the raw seeds of all Moth bean cultivars (Table 1). Subbulakshmi et al. (1976) observed that Moth bean in comparison with horse gram contained a very low level of lectin activity. Absence of lectins in Moth bean cultivars in the present investigation may reflect varietal differences as reported in soybean cultivars (Kakade et al., 1972).

The investigation indicated that compared to other common legume grains Moth bean contained considerably less of the antinutritional factors. This should reflect on overall better nutritive value of the seeds of this food legume. Saponin, also known to exert beneficial effects on lowering plasma cholesterol in man, was present in appreciable amounts. Moth bean, therefore, merits the attention of plant breeders for evolving high yielding strains of this relatively better quality legume seed for meeting dietary protein requirements in the developing world. Of the processing and cooking methods soaking appeared to be very advantageous in removing some antinutritional factors, especially phytate which was removed in substantial amounts. This traditional processing method should, therefore, be continued and encouraged in processing Moth bean. Soaking in mixed salt solution did not appear to have any significant edge over ordinary water in lowering the level of the antinutritional factors from the legume grains. Both ordinary and pressure cooking removed TIA almost completely and saponins to some extent. Sprouting had the most pronounced effect on reducing saponins. TIA was also lowered considerably but for its complete removal cooking of sprouts was necessary.

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A Study of the Desorption Isotherms of Rewetted Pigeon Pea Type-17

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ABSTRACT

Desorption equilibrium moisture content of pigeon pea type-17 was experimentally determined between 10 and 80% RH. The Haynes empirical equation was found to be the best-fit out of the seven mathematical models evaluated for 293 K, 323 K, 333 K and 353 K sigmoid-shaped isotherms. Free energy change and isosteric heat of desorption decrease with increase in moisture content between −6373 kJ/kg-mol and −525 kJ/kg-mol and between 53.429 MJ/kg-mol and 43.211 MJ/kg-mol, respectively. Ratio of latent heat of vaporization of water in pigeon pea to latent heat of vaporization of free water decreases continually from 1.168 to 1.042 over the moisture content range 5–18%, and is represented by a third-order regression equation having a correlation coefficient of 0.993.

INTRODUCTION

PIGEON PEA (*Cajnus Cajan* L.), like other foodgrains, on exposure to atmospheric air for a substantial period either adsorbs or desorbs moisture and attains an equilibrium condition depending largely upon the thermodynamic properties of the atmosphere. Brooker et al. (1974) defined equilibrium moisture content as the moisture content of the material after it has been exposed to a particular environment for an infinitely long period of time. The mechanism that controls this phenomenon is governed by water vapor pressure. A graphic representation of equilibrium moisture content of grain versus relative humidity of the environment is commonly known as adsorption or desorption isotherm. Most of the cereal grains display a hysteresis between adsorption and desorption ranging from 1 to 3 per cent. Young and Nelson (1967), Henderson (1970) and Dunstan et al. (1973) have conducted studies to compare the adsorption and desorption isotherms of foodgrains.

Pigeon pea is a popular foodgrain grown and consumed in India and its neighboring countries. However, its thermophysical and hygroscopic properties are not available in the research literature (Shafer, 1983). The knowledge of equilibrium moisture content of the foodgrain is essential for the efficient design and operational control of drying and storing systems. The objective of this study was, therefore, to establish a best-fit desorption isotherm model and also to determine quantities such as free energy change, heat of desorption and latent heat of vaporization of water in the whole pigeon pea type-17.

Many mathematical models have been suggested to describe equilibrium moisture content isotherms. While some of these equations are purely empirical in nature other have theoretical basis. Some of the well known equations selected for the evaluation for pigeon pea in this study are summarized in Table 1.

In equation [5] constants K_1 and K_2 are obtained from the relationship:

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$$-\Delta F = K_1 \exp(-K_2 M) \quad [8]$$

where ΔF is free energy change of sorption or desorption and given by:

$$\Delta F = RT \ln \left(\frac{P_g}{P_s}\right) \quad [9]$$

Nellist and Hughes (1973) and Brooker et al. (1974) have reviewed the isotherm equations giving their applicability range.

MATERIALS & METHODS

THE SUN-DRIED pigeon pea harvested in 1983 was stored in plastic bags until tested. To obtain an initial moisture content of about 25% dry basis for all the tests the usual practice of rewetting the grain was followed as discussed by Hustrulid (1962, 1963), Henderson (1970) and Chung and Pfost (1967). Saturated salt solutions were used to control the relative humidity from 10 to 80% (Lange and Forker, 1961). A sample of about twenty grains, placed in a nylon-net bag, was suspended in each conical flask which contained saturated salt solutions. The sealed flasks were then placed in an oven maintained at temperatures of 293°K, 323°K, 333°K and 353°K, respectively, within an accuracy of ± 0.5°K to allow the grain to attain the equilibrium moisture. A period of 6–8 wk was required for the grain samples to reach the equilibrium. A few grains in the bags maintained at 80% RH were affected by mold at 293°K and were eliminated from the test. The equilibrium moisture content of the grain was determined by placing them in an oven at 376°K to 72 hr.

RESULTS & DISCUSSION

EQUILIBRIUM moisture content at several relative humidities between 10 and 80% for each of the four temperatures selected was obtained experimentally. The trend of the equilibrium moisture content versus relative humidity plot (Fig. 1) shows a sigmoid-shape curve which rises sharply above 50 per cent relative humidity. Using the method of least squares (Scarborough, 1966), correlation coefficient (r) and regression constants for the seven selected mathematical models were evaluated and have been listed in Table 2.

In general, the Bradley equation [1] fits the data quite well over the entire range of relative humidity (10–80%) and test temperature. The correlation coefficient also varied between 0.988 and 0.998, but the difference between the experimental and computed values was larger at lower and higher relative humidities as compared to the middle range of 30–70%.

The BET equation [2] was tested over the entire range but the fit was not found satisfactory as is obvious from the results given in Table 2. But when it was tested for the relative humidity range between 10 and 55% only, it gave high correlation coefficients, showing the applicability of BET equation for pigeon pea in the lower range of relative humidity only. However, the Smith equation [3] fitted the data very well over the entire range of relative humidity selected for this study, having strong correlation coefficients for all the temperatures except for 293 K.

Applicability of the Henderson equation [4] for the grain was not satisfactory except at 293°K where the correlation between experimental and computed values was strong. Results of the evaluation of the models given in Table 2 clearly

Table 1—Seven isotherm equations tested for pigeon pea

Isotherm equation		Equation number	Reference	RH range applicable
$\ln[\ln(P_s/P_g)]$	$= \ln K_1 + M \ln K_2$	[1]	Bradley (1936)	2–90%
$(P_g/P_s)/M[1 - (P_g/P_s)]$	$= K_1 + K_2 \frac{P_g}{P_s}$	[2]	Brunauer et al. (1938)	2–50%
M	$= K_1 - K_2 \ln[(1 - (P_g/P_s)]$	[3]	Smith (1947)	50–95%
$\ln[\ln(\frac{1}{1 - RH})]$	$= \ln K_1 + K_2 \ln M$	[4]	Henderson (1952)	2–90%
$\ln[-RT \ln(P_g/P_s)]$	$= \ln K_1 - K_2 M$	[5]	Chung and Pfost (1967)	20–90%
RH	$= \exp(K_1 + K_2 \exp K_3 M)$	[6]	Chen (1971)	10–90%
$\ln P_g$	$= K_1 + K_2 \ln P_s + K_3 M + K_4 M^2 + K_5 M \ln P_s$	[7]	Haynes (1961)	—

Table 2—Values of different constants of isotherms equations evaluated for pigeon pea

S.No.	Isotherm Equation	Equation no.	Temp. °Kelvin	Constants K_1	K_2	K_3	Correlation coefficient
1.	Bradley Equation	[1]	293	2.916	0.8828	—	0.998
			323	2.223	0.8589	—	0.989
			333	2.6117	0.8619	—	0.988
			353	2.699	0.8666	—	0.992
2.	BET Equation	[2]		(i) Considering all experimental points (10–80% RH)			
			293	4.227×10^{-3}	0.1993	—	0.935
			323	2.035×10^{-2}	0.2639	—	0.980
			333	1.436×10^{-2}	0.2356	—	0.976
			353	1.225×10^{-2}	0.2328	—	0.975
				(ii) Considering experimental points between 10 and 55% RH			
			293	2.977×10^{-2}	0.1233	—	0.994
			323	3.896×10^{-2}	0.2136	—	0.999
			333	3.140×10^{-2}	0.1793	—	0.984
			353	2.950×10^{-2}	0.1718	—	0.999
3.	Smith Equation	[3]	293	2.3968	11.9380	—	0.994
			323	0.7837	9.2273	—	0.998
			333	1.2670	9.9626	—	0.999
			353	1.5182	10.3370	—	0.999
4.	Henderson Equation	[4]	293	3.037×10^{-2}	1.295	—	0.999
			323	6.311×10^{-2}	1.1956	—	0.996
			333	3.958×10^{-2}	1.3270	—	0.997
			353	3.686×10^{-2}	1.3269	—	0.998
5.	Chung & Pfost Equation	[5]	293	7231487.2	0.1277	—	0.938
			323	5970583.8	0.1521	—	0.898
			333	7493565.5	0.1512	—	0.870
			353	7923592.6	0.1432	—	0.878
6.	Chen Equation	[6]	293	-2.488×10^{-2}	−2.6601	-1.234×10^{-1}	0.951
			323	−0.1705	−3.0381	−0.2501	0.871
			333	-5.808×10^{-2}	−2.8627	−0.1649	0.981
			353	−0.1319	−3.2569	−0.1985	0.860
7.	Haynes Empirical Equation	[7]	293 to 353	$K_1 = -0.8078$, $K_2 = 1.02904$, $K_3 = -0.01525$, $K_4 = 1.4625 \times 10^{-3}$, $K_5 = 2.225 \times 10^{-3}$			0.994

Table 3—Heat of desorption and net heat of desorption in MJ/kg-mol for pigeon pea

Temp avg isotherm, T_a, K	Moisture content, per cent dry basis 5	6	8	10	12	15	18
307	49.65 (47.23)[a]	50.212 (47.792)	49.255 (46.835)	48.50 (46.07)	47.664 (45.244)	46.89 (44.472)	45.844 (43.424)
328	53.429 (51.059)	50.054 (47.684)	49.319 (46.949)	47.563 (45.193)	47.056 (44.686)	44.727 (42.357)	44.557 (42.187)
347	50.184) (47.85)[a]	48.214 (45.881)	47.371 (45.038)	46.295 (43.961)	45.883 (43.555)	44.892 (42.559)	43.211 (40.378)

[a] Values in brackets correspond to the net heat of desorption.

indicate that the Chung and Pfost model is not suitable for pigeon pea. Chen (1971) explained the technique for finding three constants of equation [6]. Only three apparently reliable points at 20, 50, and 80% RH were employed and applicability of equation [6] over the entire range of relative humidity was tested. With these constants equation [6] generated exactly the same values for the three points used for the evaluation of the constants, but for the other points the variation between the computed and experimental values was quite large and the correlation between experimental and calculated values for the entire range was poor.

Out of the available empirical equations in the literature, the Haynes equation [7] was tested. Results in Table 2 indicated that the Haynes equation described the isotherms well, giving a strong correlation coefficient of 0.994, over the entire range of temperature and humidity.

Free energy change is the energy required to transfer water molecules from vapor state to the solid surface or from the solid surface to vapor state. The free energy change can be considered a measure of work done by the system to accomplish adsorption or desorption process. The free energy decreased continually with increase in moisture content. Using

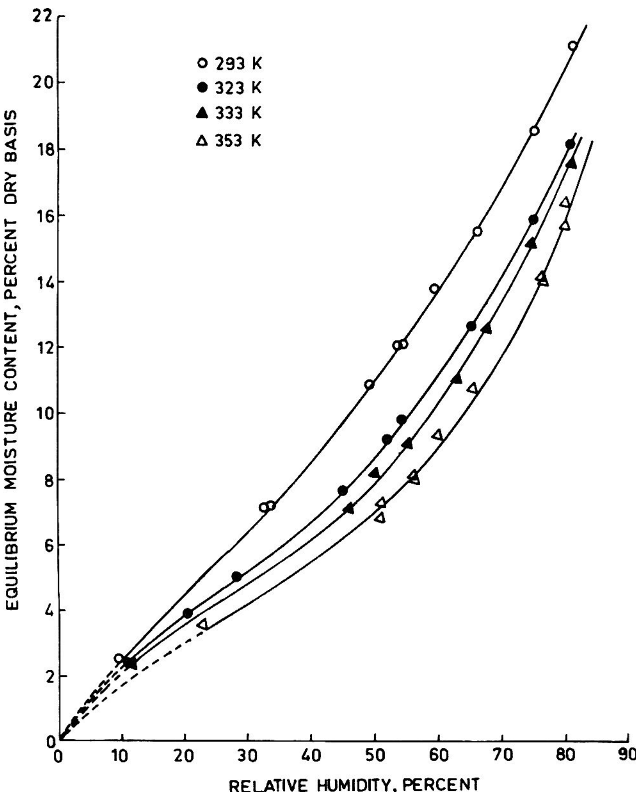

Fig. 1—Desorption isotherms for pigeon pea at several temperatures.

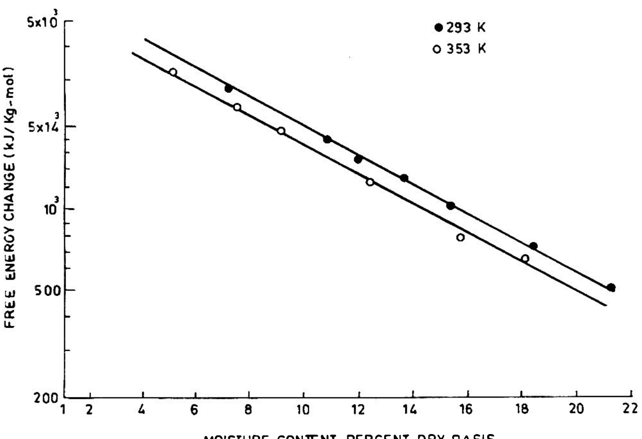

Fig. 2—Change in free energy of desorption for pigeon pea.

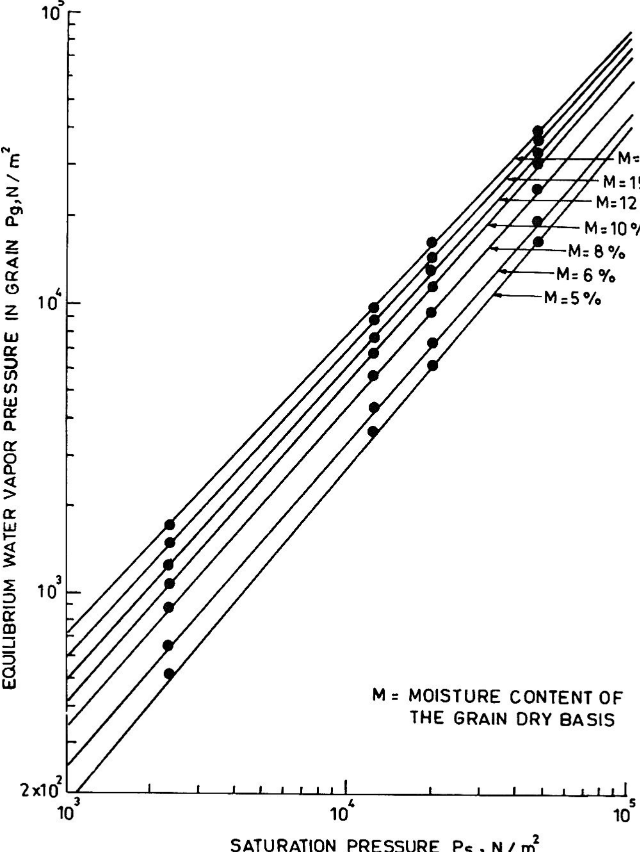

Fig. 3—Othmer plot of equilibrium moisture content data for pigeon pea.

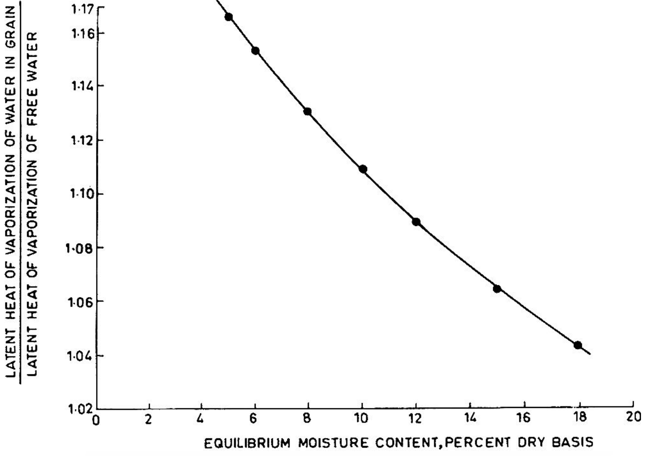

Fig. 4—Variation of the latent heat of pigeon pea and free water with moisture content.

the method of least squares constants K_1 and K_2 of equation [8] were determined for the four temperatures selected for the study. Equation [9] always gives a negative value of ΔF but for convenience a positive quantity is considered, therefore, a minus sign is used with ΔF in equation [8] to estimate the constants. The relationships between free energy and moisture content obtained are:

for 293°K

$$-\Delta F = 7231.4872 \times 10^3 \exp(-0.1277M) \quad [10]$$
$$r = 0.998$$

for 323°K

$$-\Delta F = 5970.5838 \times 10^3 \exp(-0.1521M) \quad [11]$$
$$r = 0.989$$

for 333°K

$$-\Delta F = 7493.5655 \times 10^3 \exp(-0.1512M) \quad [12]$$
$$r = 0.990$$

for 353°K

$$-\Delta F = 7923.5926 \times 10^3 \exp(-0.1432M) \quad [13]$$
$$r = 0.992$$

The plot of equations [10] and [13] is shown in Fig. 2. The other quantity determined from the isotherms is isosteric heat of desorption. The heat of desorption is that quantity of heat which is taken up during moisture migration when adsorbed moisture vapor desorbs. It is measure of the heat or energy that must be added to adsorbed gas to break the intermolecular force existing between the molecules of water vapor and the surface of adsorbent (Chung and Pfost, 1967). Isosteric heat of desorption was estimated using the expression:

$$\Delta H_{st} = R\left[\frac{T_1 T_2}{T_2 - T_1}\right] \ln\left(\frac{P_2}{P_1}\right) \quad [14]$$

If T_1 and T_2 are close together, the value of ΔH_{st} will correspond to an isotherm whose temperature is given by:

$$\frac{1}{T_a} = \frac{1}{2}\left[\frac{1}{T_1} + \frac{1}{T_2}\right] \quad [15]$$

and pressure by

$$P_a = \sqrt{P_1 P_2} \quad [16]$$

Chung and Pfost (1967) used the above expression for cereal grains and their products. The relative humidities at the constant equilibrium moisture content were converted to the equilibrium vapor pressure P_1 and P_2 and values of ΔH_{st} given in Table 3 were calculated by equation [14]. Heat of desorption decreased continually with increase in moisture content. The net heat of desorption given in Table 3 was also calculated by using the expression:

$$q = \Delta H_{st} - \lambda \quad [17]$$

In addition to the heat of desorption the latent heat of vaporization of water in pigeon pea was estimated with the help of Othmer plot, i.e., log P_g versus log P_s. As discussed by Hall (1970), the plots for constant moisture contents gave a set of straight lines (isosteres) whose slopes are related as:

$$L_g/L_s = \text{slope of the Othmer plot}$$

The correlation coefficient of the isosteres in the Othmer plot was more than 0.999. The slopes of isosteres decreased continually with increase in moisture content as shown in Fig. 3. Using the method of least squares a third-order polynomial was found to be the best-fit in the following form:

$$\frac{L_g}{L_s} = 1.265 - 2.486 \times 10^{-2}M + 1.195 \times 10^{-3}M^2 - 2.795 \times 10^{-5}M^3 \quad [18]$$

$$r = 0.998$$

The plot of equation [18] is shown in Fig. 4. The latent heat of vaporization of pigeon pea decreased continually with increase in moisture content and L_g/L_s tended towards unity as the moisture content of the grain increased.

CONCLUSIONS

THE FOLLOWING CONCLUSIONS are drawn from the study: The desorption isotherms of pigeon pea are of sigmoid-shape similar to the isotherms of other foodgrains. The Haynes equation [7] describes the isotherms with high correlation coefficient over the entire range of temperature and humidity tested for pigeon pea.

The heat of desorption, net heat of desorption, and free energy change decrease continually with increase in moisture content. The heat of desorption is consistently higher than the heat of vaporization of pure water over the entire range of moisture content tested.

The latent heat of vaporization of water in pigeon pea decreased with increase in moisture content and a third-order polynomial correlates the ratio of latent heat of vaporization of water in the grain and latent heat of vaporization of free water with moisture content.

NOMENCLATURE

Symbol	Quantity	Units
ΔH_{st}	isosteric heat of desorption	J/kg-mol
K_1, K_2, K_3, K_4, K_5	constants of isotherm equations	
L_g	latent heat of vaporization of water in grain	J/kg
L_s	latent heat of vaporization of free water	J/kg
M	moisture content of grain, percent dry basis	
P_g	partial or equilibrium pressure of water vapor in grain	N/m^2
P_s	saturation pressure	N/m^2
P_1, P_2	equilibrium vapor pressures at temperatures T_1 and T_2, respectively	N/m^2
R	universal gas constant	J/kg-mol.K
RH	relative humidity, decimal	
T	temperature	K
T_a	temperature of an average isotherm, equation [15]	K
T_1, T_2	temperatures of the adjacent isotherms	K
ΔF	free energy change	J/kg-mol
λ	heat of vaporization of free water at specific temperature	kJ/kg

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Sensory and Chemical/Physical Quality Criteria of Frozen Peas Studied by Multivariate Data Analysis

MAGNI MARTENS

ABSTRACT

Ninety-six batches of peas (*Pisum sativum* L. var. *medullare* Alef.) chosen to represent many different types of quality variations, were analysed for 11 sensory and 11 chemical/physical variables. Mealiness, hardness, fruity and sweet flavor were found to be relevant sensory quality criteria by Principal Component Analysis and Analysis of Variance. These internal quality criteria seemed to describe a type of variation different from external quality variables such as color, appearance and size. About 60% of the total variation in the sensory variables could be predicted by the chemical/physical measurements as modelled by a two-block Partial Least Squares Regression method. Alcohol insoluble solids contributed the most, especially to describe the "water" variation represented by mealiness and juiciness. The sensory and chemical/physical quality criteria were interpreted in terms of quality variations in the material as mainly due to maturation and growing seasons.

INTRODUCTION

SENSORY QUALITY is important for the success of green peas in the market. In a study by Schutz *et al.* (1984) consumers rated flavor and then texture as the most important sensory attributes with respect to purchase and use of peas, while color and appearance played less important roles. Hårdh (1982) stressed the importance of using internal sensory criteria like flavor and texture as a basis for classification of vegetables, not only external criteria such as appearance and size.

In quality control and classification of vegetables, there is a need to have objective methods for quality determination. With respect to measure sensory quality, a set of objective sensory quality criteria that describe the largest, most relevant and most reliable variations for a given product type is required.

Chemical and physical methods for indirectly determining the quality of peas for processing were explored by Ottosson (1958). Alcohol insoluble solids, dry matter, sugar content and tenderometer value were found to be relevant for measuring growth and maturity of peas for canning and freezing. However, the relationship of these chemical measurements to the sensory perception has been given little attention in the literature. In early research on pea quality sugar analysis was related to sensory quality (Blanchard and Maxwell, 1940). More recently the ability to estimate maturity of peas by different chemical, physical and sensory methods has been studied (Rutledge and Board, 1980; Shewfelt, 1984).

Quality data from sensory measurements of biological material are complex and multivariate by nature. Calibration of chemical and physical methods to predict sensory response confronts us with even more complex data-analytical problems. Many different multivariate data techniques have been developed to handle large and complex data matrices (Martens and Russwurm, 1983). Preliminary studies have shown multivariate techniques to be well suited for the examination of the total sensory variation in pea data (Hildrum *et al.*, 1984) and for the study of covariation between two types of quality measurements (Martens and van der Burg, 1985).

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Table 1—Number of pea-batches analysed each year grouped according to their tenderometer-values (TV groups)

TV groups	Years 1981	1982	1983	Total
TV1 (72-105)	12	6	6	24
TV2 (106-120)	8	4	10	22
TV3 (121-141)	5	8	8	21
TV4 (142-200)	9	10	10	29
Total	34	28	34	96

The first purpose of this paper was to establish sensory quality criteria for frozen peas. The second purpose was to study the ability of existing and traditional chemical and physical measurements to predict the sensory quality. Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression were tried out on each of the problems respectively.

MATERIALS & METHODS

Materials

Ninety six batches of wrinkle-seeded vining peas (*Pisum sativum* L. var. *medullare* Alef.) from the 1981, 1982 and 1983 seasons were analysed. The number of batches analyzed each year, grouped according to their tenderometer-values, are shown in Table 1. Twenty-two different varieties collected from three different locations in Norway, were included in the study. The batches were selected to cover a wide range of variation of green peas with respect to varieties, harvesting time and environmental factors (different growth locations and seasons). Directly after harvesting the peas were blanched (85–90 °C, 2–4 min), frozen and stored 1–2 weeks (−20 °C) before the analyses started.

Sensory analysis

The evaluation took place at the Norwegian Food Research Institute. The pea samples were steam heated on a boiling water bath and served hot (about 50 °C) to a trained profile panel consisting of 11–12 persons. The judges were women (age 30–60) selected from the local district and paid to do the sensory work. Selection of judges was based on high reproducibility and high ability for identification and verbal expression of quality properties. Three replicates of each batch were served in a randomized order within each replicate and with respect to each judge. Nine to twelve samples were evaluated in each session (i.e., 1 hr including a short break) for 11 sensory attributes (Table 2).

In 1981 a list of about 60 color, appearance, flavor and texture terms were collected from the literature (Martens *et al.*, 1983a), from agriculturists and from the judges themselves. Through discussion and training sessions using selected pea-samples, this list was reduced to the 11 terms shown, i.e., the judges were asked to group terms from the original list that expressed the same type of sensory variation and to select one term representative for each type (a mental "factor analysis"). Internal quality variables were emphasized. The same reduced list was used each year except for 1981, when only comments but no quantitative descriptions were made on off-flavor. The color term was defined as the total impression of uniformity and green strength (1 = not uniform, weak color; 9 = uniform, strong color). For appearance, the degree of even size and absence of cracks or splits was judged (1 = low degree; 9 = high degree), while the other descriptive terms followed intensity scales (1 = low intensity; 9 = high intensity). Fruity flavor was defined as the characteristic aromatic, rich and light

TABLE 2—Means and standard deviations for each of the sensory and chemical/physical variables for the certain number of batches (n) included each year and within each tenderometer-value (TV) group

	Years				TV groups				
Variables	1981 (n = 34)	1982 (n = 28)	1983 (n = 34)	F-value[a] Sign. level[b]	TV1 (n = 24)	TV2 (n = 22)	TV3 (n = 21)	TV4 (n = 29)	F-value[a] Sign. level[b]
Sensory variables									
Color	5.5 (1.1)	4.9 (1.2)	5.7 (1.0)	3.8*	5.5 (1.3)	5.7 (1.2)	5.4 (0.9)	5.1 (1.2)	1.4
Appearance	5.4 (0.5)	5.1 (1.0)	5.4 (0.6)	1.6	5.1 (0.8)	5.3 (0.7)	5.2 (0.7)	5.6 (0.6)	3.3*
Sweet flavor	4.9 (1.1)	4.5 (0.9)	4.5 (0.9)	2.5	5.4 (0.8)	5.1 (0.7)	4.4 (0.7)	3.7 (0.8)	24.2***
Fruity flavor	3.3 (1.0)	3.2 (1.1)	2.9 (0.8)	1.3	4.0 (0.9)	3.6 (0.8)	2.9 (0.6)	2.3 (0.6)	29.6***
Earthy flavor	2.4 (0.8)	2.7 (0.8)	2.6 (0.7)	1.1	1.2 (0.5)	2.2 (0.5)	2.8 (0.5)	3.2 (0.6)	31.6***
Off-flavor	—[c]	1.6 (0.4)	1.8 (0.5)	—	1.6 (0.4)	1.6 (0.3)	1.7 (0.3)	1.9 (0.7)	1.6
Hardness	3.8 (0.9)	4.0 (0.9)	3.8 (1.0)	0.3	3.1 (0.4)	3.4 (0.4)	3.9 (0.6)	4.9 (0.6)	63.9***
Skin strength	4.5 (0.8)	4.6 (0.7)	4.9 (0.7)	3.2*	3.8 (0.5)	4.5 (0.5)	4.8 (0.5)	5.4 (0.4)	57.7***
Mealiness	3.3 (1.5)	4.8 (1.1)	3.7 (1.3)	9.7***	2.4 (1.1)	3.2 (0.9)	4.4 (1.0)	5.2 (0.8)	43.3***
Juiciness	4.4 (1.1)	3.7 (1.0)	4.2 (1.2)	3.2*	5.2 (0.9)	4.7 (0.7)	3.8 (0.7)	3.0 (0.6)	47.7***
Aftertaste	1.9 (0.3)	2.5 (0.5)	2.7 (0.5)	33.0***	2.0 (0.4)	2.2 (0.5)	2.6 (0.4)	2.6 (0.7)	8.5***
Chemical/physical variables									
Tenderom. value	121.7 (30.0)	135.3 (28.3)	129.0 (26.8)	1.8	95.1 (7.5)	113.1 (4.7)	132.3 (7.1)	164.0 (14.4)	—
AIS[d], %	15.3 (2.7)	17.6 (3.0)	15.8 (2.7)	5.8**	13.3 (1.9)	14.5 (1.5)	16.8 (1.7)	19.3 (1.9)	59.1***
Soluble solids, %	9.4 (1.1)	9.0 (0.8)	9.7 (1.3)	3.5*	9.6 (0.9)	9.7 (1.0)	9.1 (0.8)	9.2 (1.4)	2.1
Dry matter, %	22.7 (2.3)	23.9 (2.5)	22.8 (2.3)	2.2	20.9 (1.7)	21.9 (1.5)	23.2 (1.4)	25.6 (1.6)	46.5***
Sucrose, g/100g	7.5 (2.0)	8.1 (1.5)	7.3 (1.2)	2.3	9.1 (1.4)	7.9 (1.4)	7.2 (1.1)	6.5 (1.3)	20.4***
Glucose, g/100g	0.6 (0.3)	0.2 (0.5)	2.2 (0.3)	242.7***	0.9 (0.1)	1.4 (1.0)	1.0 (0.8)	1.1 (0.9)	1.2
Fructose, g/100g	0.2 (0.1)	0.2 (0.1)	0.4 (0.1)	103.2***	0.2 (0.1)	0.3 (0.1)	0.3 (0.1)	0.2 (0.1)	0.2
Starch, g/100g	4.8 (2.5)	5.1 (1.6)	4.8 (1.7)	0.2	3.4 (1.8)	4.2 (1.5)	5.3 (1.6)	6.3 (1.6)	15.7***
Protein, gN/100g	0.8 (0.1)	1.0 (0.1)	1.1 (0.1)	68.2***	0.8 (0.2)	0.9 (0.1)	1.0 (0.2)	1.0 (0.2)	5.0**
Yield, kg/daa	536 (122)	644 (189)	651 (128)	4.5*	536 (166)	609 (110)	650 (130)	640 (180)	2.0

[a] One-way ANOVA of the batches between years and TV groups respectively
[b] *p < 0.05, **p < 0.01, ***p < 0.001; rest not significant.
[c] Missing value
[d] Alcohol insoluble solids

flavor of peas that may exist in addition to, but independent of sweetness, while earthy flavor represented the heavy part of the characteristic pea flavor. Off-flavor was defined as bitter, metallic, harsh flavor, while aftertaste constituted the flat, unbalanced, sweet taste that may be perceived after swallowing.

Chemical and physical measurements

Samples used for the sensory assessment were also analyzed by chemical and physical methods (Table 2). In addition different size-fractions within each batch were registered. All the chemical analyses were performed in duplicate.

Alcohol insoluble solids (AIS) were analyzed according to an AOAC method (AOAC, 1980). Soluble solids (water soluble, "Brix number") were measured at 20 °C with an Abbe refractometer (Hilger & Watts). Dry matter was determined by drying under vacuum at 70 °C overnight. Sucrose, fructose, glucose and starch were determined by a high-pressure liquid chromatography-method (Conrad and Palmer, 1976). Prior to this analysis, the starch was enzymically hydrolysed to glucose (Skrede, 1983). Protein was analysed by Kjeldahl-N. The tenderometer-value (TV), conventionally used to express the maturity of the peas, was measured on a Tenderometer (National Canners Assoc.). The size of the peas was determined after sieving each batch, according to the following size-fractions: below 7.8 mm, 7.8–8.2 mm, 8.3–9.0 mm and above 9.0 mm.

Data analysis

Means, standard deviations and Analysis of Variance (ANOVA) were included in the data treatment to give an indication of the variation and the error levels in the data.

To study the main tendencies of variation among the sensory variables, Principal Component Analysis (PCA) (Cooley and Lohnes, 1971) was performed for each year and for the three years simultaneously. The essential relationships between many sensory variables (Y-block) and many chemical and physical variables (X-block) were studied by a method of Partial Least Squares (PLS) regression on latent variables (Wold *et al.*, 1983). This method extracts a few linear combinations (PLS-factors or latent variables) of the chemical and physical data that predict as much as possible of the systematic variations in the table of sensory data.

The statistical concepts of the multivariate methods are described elsewhere (Martens *et al.*, 1983b; Martens and Martens, 1986). In both PLS and PCA the data are modelled in terms of a few "significant" factors plus residuals. The factors span the main phenomena or systematic variabilities found in the data. The residuals represent variabilities in the data that do not fit the linear model; they can be due to measurement errors, strong nonlinearities, etc. Each factor is characterized in terms of factor loadings for all the variables and factor scores for all the batches. A high factor loading means that the variable is strongly correlated to and hence well described by this factor. The sign of the loading shows if the relationship is positive or negative. A high factor score means that the object strongly contribute to span this factor. Positive scores mean higher-than-average levels of this factor, while negative scores mean lower-than-average levels. Percent explained variance for the different variables expresses what variation is modelled after a given number of factors; the remainder represents the percent residual variance.

One factor is estimated at a time. Each factor is orthogonal (totally uncorrelated) to the previous ones. Some validation technique, in this case cross-validation (Wold, 1978), is used to determine the maximum number of significant factors. This ensures predictive validity, guarding against over-fitting (modelling noise phenomena in the data).

Results from the multivariate data analytical methods are presented here in figures for the factor loadings and factor scores, and in tables for the percent explained variances (see RESULTS & DISCUSSION). Each variable was standardized to variance 1.0 prior to the multivariate analyses to ensure all the variables equal probability of participating in the modelling.

All the statistical analyses were done on a NORD-100S minicomputer (Norsk Data, Norway). The PCA and PLS calculations used a modified SIMCA 3B-program (Wold, 1981, modified by H. Martens, Norwegian Food Research Institute).

RESULTS & DISCUSSION

Input data

The values for each sensory (panel mean) and chemical/physical variable averaged over replicates for each of the 96 batches provided input data for the multivariate data analyses.

The input data were further averaged over batches and presented in Table 2, to give a general impression of the main variation in the material as expressed by the univariate ANOVA: All the sensory and chemical/physical variables showed significant differences either between years or TV-groups or both. The variation between TV-groups indicated a large quality variation due to maturation of the peas.

The univariate results thus indicated a significant variability in the material. The multivariate data analyses were then applied to gain information about the systematic. latent phenomena creating relationships between the different variables.

Table 3—Results from three-way ANOVA (batches × judges × replicates) and PCA used to explore sensory quality criteria in the 96 pea batches

Sensory variables	Mean squares from ANOVA (average for 3 years) Batches (32 d.f.)	Error (660 d.f.)	% Explained variances after PCA-factor 1	2	3
Color	45.1	1.0	31.6	80.1	84.3
Appearance	16.4	0.9	0.0	81.6	81.6
Sweet flavor	33.4	0.9	79.1[a]	79.9	84.8
Fruity flavor	30.9	0.9	88.4	89.0	90.3
Earthy flavor	18.0	0.9	93.3	93.3	93.5
Off-flavor	7.1	0.6	41.6	49.9	87.7
Hardness	28.6	0.6	62.9	83.4[b]	83.6
Skin strength	18.2	0.6	67.6	85.4	86.6
Mealiness	57.6	1.1	77.2	78.3	78.5
Juiciness	41.9	0.8	91.2	91.9	93.5
Aftertaste	6.8	0.9	47.6	49.7	89.0
Total % explained			61.9	78.4	86.7

[a] ___ interpreted as relatively very large
[b] ..... interpreted as relatively large

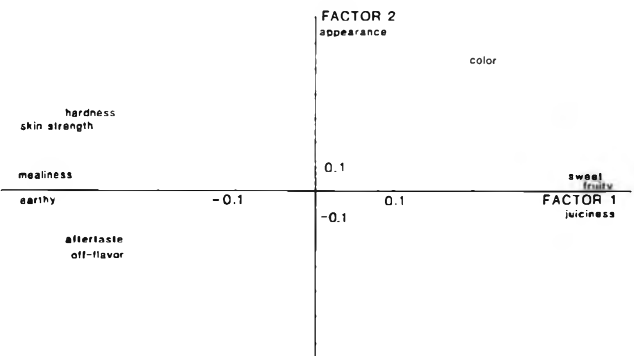

Fig. 1—Description of the main tendencies of variation in sensory quality: PCA loadings for factor 1 and 2 for 11 sensory variables analysed on 96 pea batches.

Sensory quality criteria

Results from PCA of the 11 sensory variables showed three interpretable factors which described about 87 % of the total variation in the 96 batches (about 62, 17 and 8 % respectively; Table 3). The factor loadings for factor 1 and 2 are shown in Fig. 1. Factor 1 was mainly a flavor/juiciness factor, i.e., sweet, fruity flavor and juiciness gave high and opposite loadings to earthy flavor and mealiness. In addition, the mechanical texture expressed by hardness and skin strength was correlated to factor 1. The two latter variables also played a smaller role in factor 2, which was mainly a color/appearance factor. The flavor terms off-flavor and aftertaste spanned a third but less important dimension of variability (Table 3). The small contribution to factor 3 from sweet flavor was probably related to aftertaste-sweetness and not to fruity-sweetness, thereby separating the two types of sweetness.

Sensory quality variables that (1) discriminate well between the batches, (2) do not give redundant information, and (3) have low measurement-noise, i.e., is clearly understood by the judges, should be chosen as quality criteria. Thus, results from both ANOVA and PCA as shown in Table 3 and Fig. 1 should be taken into consideration. Mealiness and juiciness both discriminated satisfactorily between the batches (see ANOVA in Table 3), but they gave apparently redundant information (contributed to the same factors in the PCA). Although mealiness showed a slightly higher noise level (error in Table 3), it is often used to describe pea quality and may thus be chosen to represent this texture dimension. In analogy, hardness seemed to describe the same tendencies of variation as skin strength in the material; only one of these two variables is probably needed. Fruity and sweet flavor gave slightly different information (see factor 3 in PCA), while earthy flavor came out as the opposite of fruity flavor. Aftertaste and off-flavor showed relatively low variation in the material and were also rated relatively low (see Table 2), indicating that the material had not been exposed to any extreme postharvest handling.

Thus, mealiness, hardness, fruity and sweet flavor were found to be relevant and representative internal sensory quality criteria for normally treated green peas. These criteria seemed to describe a different type of variation than the external color and appearance variables. These main conclusions were also drawn after each year analysed separately, thus giving confidence that the results were not caused by just random yearly fluctuations.

Table 4—Results from the PLS regression used to study relationships between the sensory (Y-block) and chemical/physical (X-block) variables. Percent explained variances for the first three main factors as well as after the six significant factors are given

Variables	% Explained variances after factor 1	2	3	6
Color	9.2	13.8[b]	17.6	27.4
Appearance	3.8	6.8	14.2	14.2
Sweet flavor	58.7[a]	59.8	71.8	71.8
Fruity flavor	62.4	62.4	67.4	69.8
Earthy flavor	66.8	66.8	68.7	69.5
Off-flavor	12.3	22.1	22.8	33.9
Hardness	71.7	71.7	75.3	79.0
Skin strength	71.2	75.1	75.1	80.0
Mealiness	75.2	76.5	76.5	81.6
Juiciness	79.6	80.8	80.8	83.7
Aftertaste	28.4	46.1	46.1	51.6
Total % explained (Y-block)	49.0	52.9	56.0	60.2
TV	83.4	83.4	85.2	91.2
AIS	90.3	90.7	92.0	96.8
Soluble solids	7.3	48.8	86.8	95.9
Dry matter	80.0	80.0	90.3	94.5
Sucrose	51.0	51.0	58.9	85.4
Glucose	0.0	68.7	78.6	92.8
Fructose	0.0	74.0	78.6	86.8
Starch	53.5	54.6	54.6	95.7
Protein	20.8	41.2	48.3	92.8
Yield	0.0	8.7	12.2	97.8
Total % explained (X-block)	38.6	60.1	68.6	93.0

[a] ___ interpreted as relatively very large
[b] ..... interpreted as relatively large

Predicting sensory quality from chemical and physical measurements

The PLS regression of the 11 sensory Y-variables versus 10 of the chemical and physical X-variables, including the harvest yield, over the 96 pea batches revealed six significant factors with which 60 % of the total variation in the sensory variables was explained (Table 4). The texture variables, sweet and fruity flavor were best explained (in average about 77%). The first three factors were most important with respect to reflecting basic variations in the material, probably due to maturation and growing seasons, as already indicated in the input data. These factors will be discussed, therefore, in more details. Factor loadings of the different variables from factor 1 and 2 and from factor 1 and 3 are plotted against each other in Fig. 2a and b respectively, and explained variances after the three first factors, and in the total after six factors, are referred to in Table 4.

Factor 1 represented mainly a texture/flavor variation. AIS contributed most strongly to this factor, closely followed by TV and dry matter describing mainly the "water" variation in peas, i.e., juiciness and mealiness, but also a "solid texture" variation represented by hardness and skin strength. In addition, a "flavor" variation dominated by sucrose positively related to sweet and fruity flavor appeared in factor 1. Earthy flavor was also included in this dimension, negatively correlated to sweet and fruity flavor as in the PCA-solution.

In the second PLS factor soluble solids, glucose and fructose were positively related to off-flavor and aftertaste. In factor 3 soluble solids together with sucrose positively related, accounted for a small variation especially in sweetness, but also in fruity flavor. From this it would seem that aftertaste-sweetness was related to soluble solids, while sweetness in connection with fruity flavor was best predicted by sucrose. With respect to the texture variables a certain covariance between dry matter and hardness was also found in factor 3, thus separating the "water" variation from the "solid texture" variation.

Color and appearance were generally not well explained by the present chemical/physical variables. The total variation in color that was described in the PCA-solution (Fig. 1) seemed to be weakly and positively related to the sugars/soluble solids variation in factor 2 and 3, while a small amount of the total variation in appearance was positively related to the dry matter in factor 3. While starch partly contributed to the flavor variation and partly to the texture variation, protein did not contribute to any specific sensory dimension, so none of these two variables seemed to be useful for predicting sensory quality.

As can be seen from Table 4, only about 4% of the variation in the Y-block was explained in the three last factors; the main contribution to these factors came from the relationships between the rest-variance in the X-block to yield. This means that the yield cannot be used as a criterion for internal sensory quality, but it is related positively to color to a small extent. In another PLS analysis without yield as an X-variable, the same solution as described in Table 4 was found but with no further explained color variance after the three first significant factors.

Relationships between sensory quality and pea size were studied by including the four different size fractions in an X-block analogous to the one in Table 4 and repeating the PLS analysis. This resulted in a better explanation of color and appearance in factor 2 from 13.8% and 6.8% (Table 4) to 30.0% and 13.3% respectively. The highest size fraction, i.e., the largest peas, contributed the most, showing a positive correlation to color and appearance, while the small peas showed a weaker and opposite relation.

Since the tenderometer-value (TV) is commonly used to express quality of peas, the ability of TV to predict the internal sensory quality was especially studied. A separate PLS analysis with TV as the only X-variable and the 9 internal flavor and texture variables in the Y-block showed that after the one significant factor, about 67% of the main texture variation and 50% of the main flavor variation were explained by TV. This means that TV alone was a relevant but not an adequate predictor of internal sensory quality.

The relationships found may be summarized as follows: (1) a "water" variation represented by juiciness negatively and mealiness positively was related mainly to the AIS content; (2) a "solid texture" variation represented by hardness was related positively to the dry matter and starch content; (3) a "sugar" variation represented by fruity/sweet flavor was related positively to the sucrose and negatively to the starch content; (4) an "aftertaste/sweet/off-flavor" variation related positively to the soluble solids/fructose/glucose content; (5) a "color and appearance" variation was only weakly related to the chemical/physical variables measured, especially the size-fractions.

VALIDATION & INTERPRETATION

Relationships between sensory and chemical variables interpreted with respect to maturation

Both from the one-way ANOVA on TV-groups (Table 2) and from the PLS scoring plot (Fig. 3), it appears that the main variation in the material was caused by maturation described by factor 1 (Fig. 2). In Fig. 3 the batches are marked according to which TV-group they belong. Confidence in the ability of the different chemical analyses to predict sensory quality can be obtained by interpreting each batch in correspondence with the loading plots (Fig. 2a, b) and checking the results with the raw data. The batches in Fig. 3 marked with dark circles, i.e., with low TV, had positive and higher-than-average scores of factor 1. This indicates that these batches had lower AIS, dry matter and higher sucrose than the average corresponding to higher juiciness, sweetness and fruitiness and lower mealiness, as also measured by the sensory panel (Table 2). The opposite tendencies were found for the "triangular" marked batches, i.e., with high TV. Thus, as maturation proceeds, the peas become more mealy and hard and less juicy, sweet and fruity, while variation in color and appearance seemed to play a less important role. In Table 2 a tendency for lower total impression of color at high TV than at low is evident. This may be due to a loss in green color, overshadowing a more homogenous

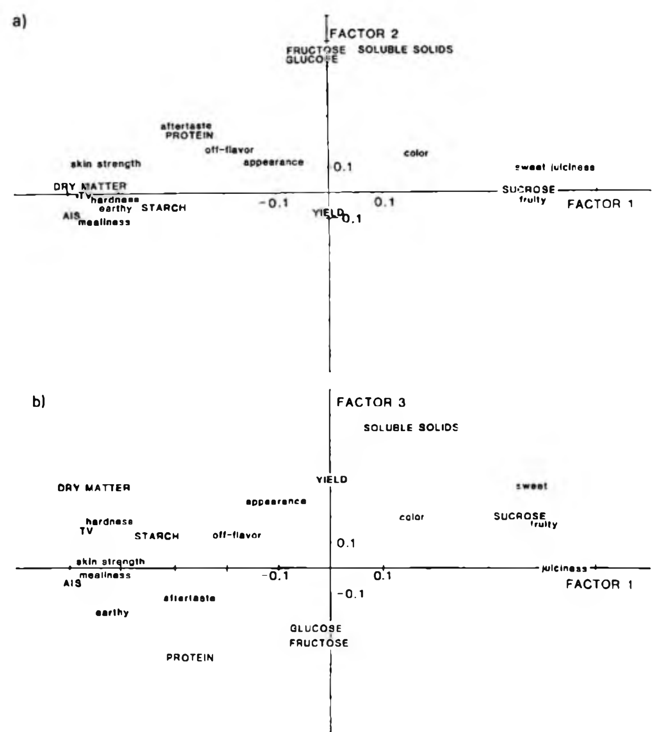

Fig. 2—Description of relationships between 11 sensory and 10 chemical/physical variables analysed on 96 pea batches: a) PLS loadings for factor 1 and 2. b) PLS loadings for factor 1 and 3.

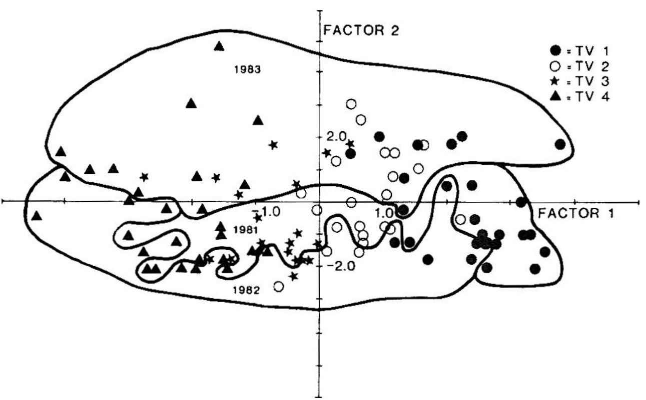

Fig 3.—Description of the relative position of the 96 batches to the factors: PLS scores for factor 1 and 2. The 96 pea batches are marked according to their tenderometer-value (TV): TV1 = 72-105, TV2 = 106-120, TV3 = 121-141, TV4 = 142-200; i. e., indicating a variation due to maturation along factor 1. Batches from each year are separated by lines indicating a variation due to seasonal differences along factor 2.

color impression. The increase in appearance with maturity is explained by getting more equal-sized and homogenous looking samples. Thus, none of the varieties included showed any marked splitting which might have reduced the appearance at high TV.

Among the chemical methods AIS showed the clearest changes in maturity as also reported by Rutledge and Board (1980) and Ottosson (1958). Among the sugars, sucrose decreased with maturity corresponding to a decrease in sweet and fruity flavor (Table 2). Blanchard and Maxwell (1940) reported that total sugar and sensory sweetness were equally related to maturity. Many authors have studied the changes in starch with maturation (Ottosson, 1958; Nielsen *et al.*, 1947). Our data revealed that starch increased significantly with maturation (Table 2) indicating less sweet and fruity flavor and harder peas. However, compared with AIS, dry matter and sucrose, starch measured by the method described in this paper, was a less reliable quality predictor. With respect to protein a small significant increase with maturity was found as also reported by Nielsen *et al.* (1947) and Ottosson (1984).

Relationships between sensory and chemical variables interpreted with respect to yearly variations

The relationships found in factor 2 (Fig. 2a) may be due to the between-year variations in the material. The PLS scoring plot in Fig. 3 indicates 1983 as different from the two other years along factor 2. This may be explained by the high glucose/fructose/soluble solids vs. off-flavor/aftertaste most strongly separating the 1983-data, as also can be seen from Table 2.

Modelling relations between variables measured together over a long period of time creates the risk of relating erroneous drift in one measurement type to erroneous drift in another measurement type. To check if the modelled relationships indeed reflect true pea quality variations instead of methodological drift being incidently correlated over years, the within-year variations were analysed separately. This confirmed that the 1983 data behaved slightly different from the two previous years. Nevertheless the AIS/TV/dry matter/sucrose vs sweet/fruity/earthy flavor and the sensory texture variables showed the same general trends all three years as described in factor 1 and 3 (Fig. 2a, b). Thus, confidence in the ability of the mentioned chemical/physical methods to predict the internal sensory quality was obtained. The relationships between the other chemical/physical measurements and sensory quality varied among the years, meaning that these variables cannot be reliable for prediction of quality from one year to another.

While the rainfall during the growing and harvesting period was normal in 1981 and 1982, there was slightly less rain in 1983. Thus, dry weather may have influenced the flavor of the peas giving a tendency towards sweet peas connected with flat, cloying aftertaste without the fresh, light and aromatic, fruity dimension. The changes in dry matter with years of extreme variation in rainfall as studied by Ottosson (1958) were not found in our data probably because precipitation during parts of the harvesting period was not as extreme as in the mentioned study.

Relationships between sensory and chemical variables interpreted with respect to varieties

No systematic variation between the twenty-two varieties was found along factor 1 and 2 (Fig. 2a). Thus, the quality criteria found to cover the variation caused by maturation and growing seasons seemed to be valid independent of variety. However, the small covariation between sensory and chemical variables described in factor 3 (Fig. 2b and Table 4) may partly be explained by differences between "extreme" varieties. A PLS scoring plot of factor 1 and 3 (not shown here) separated batches of one variety (Puget) from another (Citrina) by the higher soluble solids, sucrose and dry matter versus higher sweetness and better appearance measured in the first batches. Only small differences between varieties measured by chemical methods were reported by Ottosson (1958).

While all the varieties were represented by different tenderometer-values and most of them analysed each year, only a few were grown at different locations. From the few observations done in this study, no systematic variation between batches from different locations was found, probably because no extreme environmental factors dominated any of the sites. However, the influence of climatic and other agronomical factors on the sensory quality of peas need to be explored in another study.

CONCLUSIONS

SENSORY and chemical/physical quality criteria of frozen peas, with emphasis on internal quality, have been established: Mealiness, hardness, fruity flavor and sweetness together were found to be relevant for describing the total internal quality variation in 96 representative pea batches. About 77% of this variation could be predicted by a combination of different chemical/physical measurements; AIS, dry matter, TV and sucrose contributed the most. TV alone correspondingly accounted for about 58%.

These quality criteria obviously reflected a variation in the material mainly due to maturation. A less dominating "aftertaste/sweet/off-flavor" variation related positively to soluble solids/fructose/glucose content was interpreted as due to yearly variations. A variation due to varieties was small and generally difficult to distinguish from the effect of maturation.

A significant variation in the external color and appearance variables in the material could only weakly be related to the size of the peas and to the product yield, and none of the chemical variables nor TV were found to be important in this connection.

Multivariate data analysis has been an important tool for exploring relationships within one block (PCA) and between two blocks (PLS) of many variables.

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Essential Elements and Cadmium and Lead in Fresh and Canned Peas (*Pisum sativum L.*)

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ABSTRACT

Sixteen essential elements along with cadmium and lead were determined in fresh and canned peas (*Pisum sativum L.*). Samples were taken during the canning process to determine where changes in element content occurred. The concentration of each sample was compared statistically to other samples taken at different stages of the process. Canned peas contained significantly lower concentrations of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, silicon, and zinc than fresh peas. As sodium chloride was added during the canning process, higher concentrations of chloride and sodium were found in the canned product. A 100g serving of drained canned peas supplied less than 11% of the RDAs or of the estimated safe and adequate daily dietary intakes except for chloride and sodium. Retention of all elements, except chloride and sodium, ranged from 24–86%.

INTRODUCTION

PREVIOUS WORK done on the elemental content of peas has included mostly macro-elements such as calcium, iron, phosphorus, potassium and sodium (Ensminger et al., 1983; Anonymous, 1962; Watt and Merrill, 1963). Work has been published on other elements but little has been done to show the effects of canning on them.

The objectives of this research were to determine and compare the concentration of 18 elements, including cadmium and lead, in fresh peas with peas at different stages of the canning process.

MATERIALS & METHODS

Sampling

Samples of fresh, before blanch, after blanch and canned peas (*Pisum sativum L.*) were obtained at the KMC Corporation plant in Queen Anne, MD. The peas (Target variety) were harvested locally in the summer of 1981 and commercially canned within a few hours after harvesting. Shelled peas were received in truckloads at the processing plant, conveyed through shakers to remove any debris (fresh sample), sized, washed with a surfactant solution (sodium lauryl sulfate) to remove any dirt and inspected (before blanch sample). The peas were then blanched for 3 min at 82°C (180°F) (after blanch sample). Thirty milliliters of filling medium concentrate (solution of 13% NaCl and sucrose in tap water) was added to each 454g (16 oz, 303 × 406) C-enamel can. Then approximately 283g (10 oz) of peas was added, followed by approximately 140 mL (5 fl oz) of hot tap water before sealing. The cans were then placed in retorts, processed for 18 min at 124°C (256°F), and cooled in water (canned sample) before storing.

Six samples were taken at each step of the canning process throughout one day. Sampling from the line was coordinated so that fresh, in-process and canned samples were of the same batch. Six samples of filling medium concentrate and two of processing water were taken during the period.

Statistical design and analysis

Fresh, before blanch, and after blanch samples weighed 0.4–1.4 kg each. Six cans of peas were taken for each of the six canned pea and processed drained liquid samples. The canned samples were stored at 21°C (70°F) for 18 wk before being opened, drained and mixed. Preliminary analyses were performed on three canned pea samples to determine the number of samples needed to give 15% variation for a paired "t" test. As a result, six samples were chosen to keep the variation about 15%. The data were analyzed using Duncan's tests. Analyses of variance were performed at the 1% and 5% levels to test for significant differences.

Reagents

The following A.C.S. certified reagents and materials were obtained from Fisher Scientific Company (Fair Lawn, NJ) and prepared as indicated: hydrochloric acid, 37.5%; Whatman #41 ashless filter paper; nitric acid, 70%; atomic absorption standards; lanthanum oxide, 98% pure, was dissolved in water and hydrochloric acid for a 5% (w/v) solution; and cesium chloride was dissolved in water for a 1% (w/v) solution. For phosphorus and silicon determinations the following A.C.S. certified Fisher reagents were used: potassium dihydrogen phosphate to prepare a 500 ppm standard; ammonium molybdate to prepare a 10% solution; 53.5g ammonium chloride was dissolved in water, 70 mL of 30% ammonium hydroxide was added and the solution diluted to 1L; 1-pentanol; and diethyl ether. Used for the chloride determination were sodium carbonate as a 5% (w/v) solutions, silver nitrate solution, and nitric acid, 70%.

Sample preparation

Samples were prepared for analyses by mixing and pureeing with a Cuisinart food processor (Cuisinarts, Inc., Greenwich, CT), freezing at −8°C (18°F), freeze-drying on a Virtis freeze dryer for 48 hr, grinding in the food processor and storing in a freezer. Prior to weighing samples to be wet ashed, the frozen aliquots were ground with a Waring Blendor, freeze-dried again for 48 hr and stored in a desiccator. At no time were samples in contact with any metals except stainless steel.

The wet ashing procedure of Simpson and Blay (1966) was used for atomic absorption spectrophotometric analyses of all elements except chloride. Duplicate 1.00 ± 0.12g freeze-dried pea samples were analyzed.

Canned pea samples were prepared for chloride determination following AOAC Methods 3.071 (AOAC, 1980). Duplicate 1.00 ± 0.10g freeze-dried samples were moistened with 20 mL 5% Na_2CO_3 solution, dried at 90°C for 3 hr and ignited at 450°C overnight. After washing and filtering the ash with hot water, the filter papers were ashed at 450°C for 2.5 hr. The ash was dissolved in 4 mL of HNO_3 (1:4), filtered, added to the first washings and diluted to 100 mL.

Samples of plant processing water, filling medium concentrate and processed drained liquid were diluted 1:1 and contained 4% concentrated HNO_3.

Analytical methods

Elemental analyses were performed with a Perkin-Elmer Model 403 atomic absorption spectrophotometer. A 10 cm (4 in.) 1-slot burner head and standard air-acetylene flame were used for elemental analyses except for molybdenum and tin for which a nitrous oxide-acetylene burner and flame were used. Single element hollow cathode lamps were used for all elements. The instrument settings and other experimental conditions were in accordance with the manufacturer's specifications.

Appropriate dilutions were used to determine calcium, magnesium, potassium and sodium in all samples. Samples diluted for calcium and magnesium analyses contained 1% (w/v) lanthanum to overcome potential anionic interferences. Cesium chloride at a final concentration of 2000 ppm cesium was used for potassium and sodium analyses.

Duplicate aliquots of the wet-ashed solutions were used for indirect

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determination of phosphorus and silicon. Aliquots of all pea samples, plus water, filling medium concentrate, and processed liquid samples were analyzed by the procedure described by Parker (1972).

Chloride was also determined indirectly (Parker, 1972). Except for the canned peas, duplicate 0.45 ± 0.08g samples of freeze-dried peas were analyzed. Four mL of the canned pea solutions prepared by the AOAC (1980) method were analyzed by the chloride procedure as were aliquots of the processing water, filling medium concentrate and processed liquid samples.

Accuracy of analytical procedures

Analyses were done on five replicates of 0.60 ± 0.08g National Bureau of Standards (NBS) Citrus Leaves (Standard Reference Material #1572). These replicates were analyzed following the same analytical procedures used in this work. Data on the 14 elements analyzed for which NBS standards data were available showed variations of 4.4% or less for iron, magnesium, manganese, potassium, sodium and zinc; and 14.9%, 18.8%, 18.8% and 30.8% for calcium, copper, lead and phosphorus, respectively; cadmium, chromium, molybdenum, and nickel were below the detection limit of the instrument. When standard deviations were considered, there were differences of less than 9.8% between the NBS certified values and the values obtained following the procedure used in this work, except for phosphorus which was determined indirectly.

RESULTS & DISCUSSION

ALL DATA are presented on a wet weight basis. Moisture content of the pea samples was determined in duplicate on each of the six freeze-dried samples. The mean moisture content of fresh peas was 78.38% with a standard deviation of ± 1.74; before blanch moisture was 80.18% with a standard deviation of ± 1.79; for after blanch, it was 77.80% with a standard deviation of ± 1.31; and for canned peas it was 78.57% with a standard deviation of ± 0.58.

The concentration of 16 essential elements and cadmium and lead in canned peas are given in Table 1. Statistical analyses of the eleven essential elements which were above the detection limit are given in Table 2. Table 3 shows the concentration of the 16 elements and cadmium and lead in filling medium concentrate, processed drained liquid, and plant water. Percent retention of the elements is given in Table 4 and percents of Recommended Dietary Allowances (RDA) (Anonymous, 1980) and estimated safe and adequate daily dietary intakes per 100g of fresh and canned peas are presented in Table 5.

Cadmium was below the detection limit of the AAS instrumentation used (<0.004 and <0.005 mg/100g). Bielig and Treptow (1978) and Schroeder et al. (1967a) reported fresh pea cadmium values of 0.0038 mg/100g and 0.001-0.006 mg/100g, respectively. Boyer and Johnson (1982) reported a canned pea cadmium value of 0.006 mg/100g.

Calcium in canned peas (17.3 mg/100g) was significantly lower than that in fresh peas (20.2 mg/100g). This decrease was probably due to calcium leaching into the liquid. These values are comparable to those reported by Bridges (1941), Ensminger et al. (1983), Lee et al. (1982), and Watt and Merrill (1963). Fresh and canned peas are poor sources of calcium, with a 100g serving, respectively, supplying 2.5% and 2.2% of the RDA for calcium.

Due to the addition of filling medium to the canned product, chloride in canned peas (531 mg/100g) was significantly higher than the amount found in fresh peas (26.0 mg/100g). This increase is also reflected in results reported by Bridges (1941). Canned peas are an excellent source of chloride, with 100g supplying 15.6% of the estimated safe and adequate daily dietary intake.

Chromium was below the detection limit of the AAS instrumentation used (<0.01 mg/100g). Schroeder et al. (1970a) reported a chromium value of 0.362 mg/100g for fresh peas.

Cobalt was also below the detection limit of the AAS instrumentation used with <0.05 mg/100g. Fresh pea cobalt values of 0.003 mg/100g and 0.019 mg/100g, respectively, have been reported (Anonymous, 1962; Schroeder et al., 1967b).

The copper value of 0.07 mg/100g in canned peas was significantly lower than the 0.14 mg/100g value found for fresh peas. This decrease was probably due to some copper being extracted during washing and also by the can liquid, with processed liquid containing 0.03 mg/100 mL copper. Similar results have been reported by Bridges (1941), Anonymous (1979), and Pennington and Calloway (1973). Fresh and canned peas were fairly poor sources of copper, with a 100g serving supplying 5.6% and 2.8% of the estimated safe and adequate daily dietary intake for copper.

Fresh (1.97 mg/100g) and canned (1.05 mg/100g) peas were excellent sources of iron with 100g, respectively supplying 19.7% and 10.5% of the RDA for iron. There was a significant decrease in iron due to processing where some iron was extracted during washing and also by the can liquid. Lee et al. (1982) also reported a decrease in iron due to processing with a fresh pea value of 2.4 mg/100g and a canned pea value of 1.1 mg/100g.

Lead values of <0.2 mg/100g to <0.3 mg/100g were below the detection limit of the AAS instrumentation used. Boyer and Johnson (1982) reported lead values of 0.019 mg/100g and 0.043 mg/100g.

Canned peas (13.5 mg/100g) were significantly lower in magnesium than fresh peas (21.0 mg/100g). Again, the decrease in magnesium due to processing, was caused by the element being extracted into the liquid, with processed liquid containing 13.6 mg/100mL of magnesium. Magnesium values

Table 1—Concentration of elements in canned peas

Element	Fresh (mg/100g)	Before Blanch (mg/100g)	After Blanch (mg/100g)	Canned (mg/100g)
Cadmium	<0.005[a]	<0.004[a]	<0.005[a]	<0.004[a]
Calcium	20.2 ± 1.2[b]	17.5 ± 2.3	20.5 ± 2.8	17.3 ± 1.1
Chloride	26.0 ± 2.1	21.4 ± 0.9	14.0 ± 1.5	531 ± 66
Chromium	<0.01[a]	<0.01[a]	<0.01[a]	<0.01[a]
Cobalt	<0.05[a]	<0.05[a]	<0.05[a]	<0.05[a]
Copper	0.14 ± 0.02	0.11 ± 0.01	0.10 ± 0.01	0.07 ± 0.01
Iron	1.97 ± 0.30	1.35 ± 0.15	1.46 ± 0.09	1.05 ± 0.11
Lead	<0.2[a]	<0.2[a]	<0.3[a]	<0.2[a]
Magnesium	21.0 ± 1.7	19.4 ± 1.9	20.2 ± 1.9	13.5 ± 0.3
Manganese	0.38 ± 0.07	0.31 ± 0.04	0.31 ± 0.05	0.22 ± 0.02
Molybdenum	<0.2[a]	<0.1[a]	<0.2[a]	<0.1[a]
Nickel	<0.3[a]	<0.3[a]	<0.4[a]	<0.3[a]
Phosphorus	48.3 ± 4.7	42.3 ± 4.3	41.4 ± 5.6	27.2 ± 1.4
Potassium	270 ± 24	218 ± 13	204 ± 23	133 ± 8
Silicon	4.20 ± 1.41	4.32 ± 1.46	5.47 ± 1.20	1.00 ± 0.43
Sodium	2.37 ± 0.91	7.05 ± 1.85	7.60 ± 1.73	310 ± 36
Tin	<3[a]	<3[a]	<3[a]	<3[a]
Zinc	0.81 ± 0.10	0.73 ± 0.11	0.68 ± 0.08	0.58 ± 0.06

[a] Element below the detection limit of the AAS instrument. Detection limits differ from each other due to sample size and moisture content.
[b] Standard deviation.

Table 2—Statistical analyses of concentration of elements in canned peas[a]

Element	Fresh vs before blanch	Fresh vs after blanch	Fresh vs canned	Before blanch vs after blanch	Before blanch vs canned	After blanch vs canned
Calcium	*	ns	*	*	ns	*
Chloride	ns	ns	**	ns	**	**
Copper	**	**	**	ns	**	**
Iron	**	**	**	ns	*	**
Magnesium	ns	ns	**	ns	**	**
Manganese	*	*	**	ns	**	**
Phosphorus	*	*	**	ns	**	**
Potassium	**	**	**	ns	**	**
Silicon	ns	ns	**	ns	**	**
Sodium	ns	ns	**	ns	**	**
Zinc	ns	*	**	ns	*	ns

[a] Statistical differences as analyzed by Duncan's Test: ns = no significant difference at 1% and 5% levels; * = significant difference at 5% level; ** = significant difference at 1% level.

Table 3—Concentration of elements in filling medium concentrate, processed drained liquid, and plant water used in processing canned peas

Element	Filling medium Concentrate[a] (mg/100mL)	Processed drained Liquid (mg/100mL)	Plant water (mg/100mL)
Cadmium	0.02 ± 0.00[b]	<0.002[c]	<0.002[c]
Calcium	3.69 ± 0.23	8.76 ± 0.69	1.85 ± 0.57
Chloride	13,300 ± 1,130	559 ± 128	0.05 ± 0.02
Chromium	<0.002[c]	<0.002[c]	<0.002[c]
Cobalt	0.16 ± 0.02	<0.02[c]	<0.02[c]
Copper	0.01 ± 0.00	0.03 ± 0.01	<0.002[c]
Iron	0.16 ± 0.02	0.85 ± 0.04	<0.001[c]
Lead	0.17 ± 0.04	<0.05[c]	<0.05[c]
Magnesium	0.39 ± 0.04	13.6 ± 0.3	0.72 ± 0.23
Manganese	0.02 ± 0.00	0.16 ± 0.03	<0.001[c]
Molybdenum	<0.04[c]	<0.04[c]	<0.04[c]
Nickel	0.33 ± 0.04	<0.1[c]	<0.1[c]
Phosphorus	0.03 ± 0.02	11.0 ± 1.3	0.01 ± 0.01
Potassium	1.45 ± 0.05	125 ± 8	0.82 ± 0.12
Silicon	0.63 ± 0.08	<2.0[c]	2.55 ± 0.49
Sodium	4,250 ± 212	302 ± 32	7.08 ± 3.66
Tin	1.08 ± 0.13	<0.1[c]	<0.1[c]
Zinc	<0.003[c]	0.24 ± 0.02	<0.003[c]

[a] Made up by dissolving NaCl and sucrose in tap water.
[b] Standard deviation.
[c] Element below the detention limit of the AAS instrument.

Table 4—Retention of elements in canned peas, on the basis of element concentration in fresh peas being 100%

Element	Fresh vs. Canned Peas Retention (%)[a]
Calcium	86
Chloride	N.A.[b]
Copper	50
Iron	53
Magnesium	64
Manganese	58
Phosphorus	56
Potassium	49
Silicon	24
Sodium	N.A.[b]
Zinc	72

[a] Retention = canned/fresh × 100.
[b] NaCl was added during canning process.

of 35 mg/100g for fresh peas and 20 mg/100g for canned peas have been reported (Anonymous, 1979). Fresh and canned peas were good sources of magnesium supplying 6.0% and 3.9% of the RDA for magnesium with a 100g serving.

Fresh (0.38 mg/100g) and canned (0.22 mg/100g) peas were also good sources of manganese by respectively supplying 10.1% and 5.9% of the estimated safe and adequate daily dietary intake for manganese. There was a significant decrease in manganese due to processing, with the element being lost by washing and by extraction into the liquid. Fresh pea manganese

Table 5—Percentages of recommended dietary allowances and estimated safe and adequate daily dietary intakes per 100g of fresh and canned peas

Element	Fresh Peas	Canned Peas
Calcium	2.5[a]	2.2
Chloride	0.8[b]	15.6
Copper	5.6[b]	2.8
Iron	19.7[a]	10.5
Magnesium	6.0[a]	3.9
Manganese	10.1[b]	5.9
Phosphorus	6.0[a]	3.4
Potassium	7.2[b]	3.5
Silicon	c	c
Sodium	0.1[b]	14.1
Zinc	5.4[a]	3.9

[a] Recommended Dietary Allowance as set by the Food & Nutrition Board, National Research Council (Anon., 1980); % RDA in 100g serving for adult male.
[b] Estimated safe and adequate daily dietary intake (%) in 100g serving for adults (Anon., 1980). Percentages are calculated on the midpoint values of ranges of recommended intakes.
[c] No estimated safe and adequate daily dietary intake value has been recommended.

values of 0.27 mg/100g to 0.41 mg/100g have been reported (Bridges, 1941; Koivistoinen et al., 1974; Peterson et al., 1946).

Molybdenum values of <0.1 mg/100g to <0.2 mg/100g were below the detection limit of the AAS instrumentation used. Schroeder et al. (1970b) reported molybdenum values of 0.34-0.35 mg/100g for fresh peas.

With values of <0.3 mg/100g to <0.4 mg/100g, nickel was also below the detection limit of the AAS instrumentation used.

Phosphorus in canned peas (27.2 mg/100g) was significantly lower than the amount found in fresh peas (48.3 mg/100g). Again, this decrease was due to phosphorus being lost by washing and by extraction into the liquid. Similar decreases in phosphorus due to processing have been recorded by Lee et al. (1982) and Watt and Merrill (1963). Fresh and canned peas were fairly good sources of phosphorus, with 100g supplying 6.0% and 3.4% of the RDA, respectively.

Fresh (270 mg/100g) and canned (133 mg/100g) peas were also fairly good sources of potassium with a 100g serving supplying 7.2% and 3.5% of the estimated safe and adequate daily dietary intake. Potassium decreased significantly during processing due to washing and to extraction into the liquid. Comparable potassium values have been reported (Anonymous, 1962; Lee et al., 1982; Watt and Merrill, 1963).

Silicon also significantly decreased during processing, with 4.20 mg/100g of silicon in fresh peas and 1.00 mg/100g in canned peas.

The use of the sodium lauryl sulfate surfactant solution during processing had no significant effect on sodium concentration but, sodium significantly increased during processing due to the addition of filling medium to the canned sample. Fresh pea (2.37 mg/100g) and canned pea (310 mg/100g) values were comparable to values reported (Anonymous, 1962; Lee et al., 1982; Watt and Merrill, 1963). Canned peas were an

excellent source of sodium, with the product that was sampled supplying 14.1% of the estimated safe and adequate daily dietary intake for sodium.

The tin value of <3 mg/100g was below the detection limit of the AAS instrumentation used.

Zinc in fresh peas (0.81 mg/100g) decreased significantly due to processing, with canned peas containing 0.58 mg Zn/100g. Processed liquid with 0.24 mg/100mL of zinc suggested that zinc was extracted by the liquid. Similar results have been reported (Murphy et al., 1975; Anonymous, 1979). Fresh and canned peas were good sources of zinc, with 100g supplying 5.4% and 3.9% of the RDA for zinc, respectively.

SUMMARY

EXCEPT for chloride and sodium, all the elements analyzed which were within the detection limit of the instrument decreased significantly in canned peas when compared to fresh peas. This included calcium, copper, iron, magnesium, manganese, phosphorus, potassium, silicon, and zinc. These decreases were probably caused by the elements being extracted out during washing of the peas prior to blanching and/or during the actual thermal processing where elements were extracted into the liquid. Chloride and sodium contents in canned peas increased due to the sodium chloride content of the filling medium.

A 100g serving of drained, canned peas supplied less than 11% of the established RDAs or estimated safe and adequate daily dietary intakes of the elements studied, except for chloride and sodium which will vary depending on the concentration of the medium used. The concentration of elements in canned peas ranged from 24% to 86% of the amount present in the fresh product, excluding chloride and sodium which were added to the canned sample during the canning process.

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Mushroom Blanch Water Concentration by Membrane Processes

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ABSTRACT

Mushroom blanch water was concentrated by ultrafiltration (UF) and reverse osmosis (RO). UF prefiltration was essential in preventing severe fouling during the RO process. When the UF blanch water permeate was processed by RO, linear relationships between pressure and flux were observed at all concentrations tested. The blanch water was concentrated by UF/RO from 2% to 13% total solids at 60°C and 120 KPa/5000 KPa operating pressures with flux higher than 15 L/m^2 hr. Maximum concentration obtained was approximately 20% total solids with 90% recovery of the nonvolatiles. Recoveries of some major volatiles were above 50%. Panelists could not differentiate the original from the reconstituted blanch waters in sensory evaluations.

INTRODUCTION

BLANCHING is a necessary step in the canned mushroom process to soften the tissue, inactivate enzymes and destroy yeasts and molds. Blanch water contains approximately 2% solids, composed mainly of sugars, amino acids, nucleotides and aroma compounds (Wu et al., 1981). Recovery of flavoring components from mushroom blanch water was traditionally done by vacuum evaporation or steam jacket kettle concentration. Wu et al. (1981) added 4% corn starch in mushroom blanch water as flavor carrier and then dehydrated by freeze drying, drum drying and spray drying. The drum dried product was preferred by taste panels (Wu et al., 1981). However, thermal evaporation and dehydration processes are energy consuming and inevitably, would cause tremendous flavor losses, thus decreasing the quality of the final product.

Membrane concentration processes have recently received wide attention in the food industry. Due to the lower energy requirement (Delaney and Donnelly, 1977; DeBoer and Hiddink, 1980; Pepper, 1980), ultrafiltration (UF) and reverse osmosis (RO) have been studied extensively for recovering valuable food materials from waste effluents. UF was used to recover protein from effluents of slaughterhouse operations (Goldberg, 1980). Braddock (1982) recovered limonene from citrus processing waste streams by using UF and RO. Whey protein and lactose can be recovered from cheese whey by membrane processing (Morris, 1979; Horton et al., 1970). The objective of this study was to investigate the feasibility of using UF and RO to recover the usable components from mushroom blanch water, so that the waste effluent could be converted into a valuable flavoring product. Two important factors considered in this study were process performance and product quality.

MATERIALS & METHODS

Mushroom blanch water preparation

Simulated mushroom blanch water for experimental work was prepared by the following methods. Fresh mushrooms (*Agaricus bisporus*) were washed, weighed and blanched in equal weight of water at 97°C in a steam jacketed kettle for approximately 10 min. The resulting blanch water was reused three to four times for blanching other batches of fresh mushrooms until the soluble solids of the blanch water exceeded 2%. The blanch water was then standardized to 2% solids, stored at 4°C and used within 2 days after preparation.

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Equipment

The ultrafiltration processes were performed on a Romicon hollow fiber UF unit (Model HFXS-5/10, Woburn, MA) equipped with a PM-10 membrane cartridge. This cartridge provides 0.46 m^2 membrane area with molecular weight cut-off of 10,000 daltons and inside diameter of 1.09 mm. The recommended maximum operating pressures at 20, 50 and 75°C for the membrane were 276, 241, and 172 KPa, respectively. The schematic diagram of the ultrafiltration system is shown in Fig. 1.

The De Danske Sukkerfabrikker (DDS) Lab module-20 RO Unit (Copenhagen, Denmark) was used for the reverse osmosis study. The module is a plate-and-frame system and can be installed with up to 40 segments of membranes. Each segment provides 0.018 m^2 of effective separation area. In this study, however, only 12 segments of membranes which gave 0.216 m^2 effective membrane area were used. The DDS HR-98 membrane was used in this study. A similar membrane, HR-95, has been used by Kerr et al. (1984) to concentrate the extract made by solid-liquid extraction of mushroom solids. The permeability of a 0.2% NaCl solution with HR-98 membrane is less than 1.5%. The pressure and temperature limits for HR-98 membrane were 8.0 MPa and 80°C, respectively.

The RO flow system is similar to the UF system shown in Fig. 1 except the back-flush system and flow meter are not included. The permeate flux of RO was measured with graduated cylinder and stop watch.

Fouling studies

Processing parameters being tested for fouling phenomena of UF were operational temperature, pressure and feed flow velocity. The testing conditions were: transmembrane pressure, 62.06 to 124.11 KPa; feed flow velocity, 0.16 and 0.37 m/sec; temperature, 20° and 60°C. The transmembrane pressure (ΔP_T) was calculated by: $\Delta P_T = (P_{inlet} + P_{outlet})/2$.

In the case of RO, the fouling phenomena of both initial feed and the feed being prefiltered by UF were investigated. The initial feed was standardized with distilled water to the same solids (1.86%) as the UF permeate before running the tests. At fixed feed flow rate (7.3 ± 0.1 L/min) and selected combinations of temperatures (27°C and 60°C) and transmembrane pressures (3000 KPa and 4000 KPa), the permeate flux was measured at various time intervals to determine flux decay. The feed concentration was kept constant by recycling both the permeate and the retentate to the feed tank. For RO operation at 60°C, a higher concentration of feed (8.89% solids) was also tested to investigate the effect of feed concentration on fouling.

Mushroom blanch water concentration

To prevent severe fouling, the concentration process was conducted in two stages. The mushroom blanch water at 2% solids was first processed with UF, and the UF permeate was then concentrated with RO.

The processing performances were investigated at various transmembrane pressures, feed concentrations and operation temperatures. At selected feed concentration and temperature, the rate of concentration was determined by measuring the permeate flux at various transmembrane pressures of the module. As in the fouling study, constant concentration was maintained by recycling both permeate and retentate. The test conditions used in this study are given in Table 1.

After the effect of various process conditions on flux was investigated, the concentrated product was prepared by the two-stage operation as stated above. The UF was conducted at 120 KPa operating pressure and 0.43 m/sec of feed velocity, the operating pressure and feed rate for RO were 5000 KPa and 7.3 L/min. The UF and RO retentates were then mixed to form the final concentrated product. All samples, except RO permeate, were standardized with distilled water to the same solids (1.86%) as the UF permeate before analysis.

Composition analyses

Analysis of aroma compounds. Five hundred milliliters of sample containing a predetermined amount of n-nonanol as internal standard was continuously extracted with 100 mL of redistilled n-pentane in a modified Likens-Nickerson apparatus (Wu et al., 1981) for 4 hr. The pentane extract was dried with anhydrous Na_2SO_4 and concentrated by a fractional distillation column to about 1 mL. The extract was further concentrated with the aid of a small Pyrex ampoule to about 15 μL (Cronin and Ward, 1971).

Gas chromatographic analysis of the above extract was carried out in a 0.3175 cm (i.d.) × 2.4 m (length) stainless steel column packed with 10% Carbowax 20M on Chromosorb W/HP (80/100 mesh). An Aerograph Series 3700 gas chromatograph (Varian Aerograph, Walnut Greek, CA), equipped with a FID detector was used. Three microliters of sample were injected, chromatographed isothermally at 60°C for 5 min, programmed at 2°C/min to 200°C and held for 15 min. The peak area calculations were accomplished with a LDC 308 electronic integrator (Shannon International Airport Co., Clare, Ireland).

Analyses of nucleotides and sugars. Nucleotides and sugars were analyzed by the Waters HPLC system which consisted of a WISP 710B autoinjector, model 6000 A pumps, a model 440 absorbance detector, a model R401 differential refractometer, a model 720 system controller and a model 730 data module (Waters Assoc., Milford, MA). Prior to HPLC analysis, samples were purified and concentrated following the procedure of Liu and Chang (1975). The nucleotides were separated on a μ-Bondapak C_{18} column (Waters Assoc.) and detected at 254 nm (Qureshi et al., 1979). A linear gradient from 100% solvent A (water/acetic acid/PIC A, 97.5/1.5/0.2 by volume) to 100% solvent B (water/acetic acid/ methanol/PIC A, 77.5/1.5/20/ 0.2 by volume) was used as the elution solvent system. PIC A (pair-ion-chromatography reagent A) was purchased from Waters Assoc. The sugars were separated isocratically on a Lichrosorb NH_2 column (E. Merck Co., Germany) using acetonitrile/ H_2O (75/25, v/v) as eluting solvent and the refractometer as detector (Ling, 1983). The major peak on the HPLC chromatogram was used to calculate the retentivities of the membrane processes. Many simple sugars and sugar alcohols such as glucose, galactose, sorbitol and mannitol were found to have similar retention times under the above HPLC condition. Therefore, this major peak from HPLC was collected and rechromatographed by GC after evaporating the solvent and preparing the trimethylsilyl derivatives of the sugars with Tri-Sil Z (Pierce Chemical Co., Rockford, IL). Gas chromatographic analysis was performed on 3% SE-30 coated Chromosorb G/HP column; the column temperature was programmed from 160°C to 230°C at 1°C/min. The standards, IMP (inosine 5'-monophosphate), GMP (guanosine 5'-monophosphate) and sugars, were purchased from Sigma Co. (St. Louis, MO).

Proximate analysis. Total solids, crude proteins and total ash were determined according to AOAC methods (1980).

Sensory evaluation

The UF and RO retentates were mixed and the mixture was diluted to solids of approximately 2%, which was the same as that of the feed concentration. The reconstituted sample and the original mushroom blanch water were both warmed to 60°C and then submitted to the panelists for sensory evaluation. The panelists were first given freshly prepared mushroom blanch water as control and asked to evaluate the unidentified reconstituted sample and the original mushroom blanch water. Considering the control sample as good, the panelists scored the unknown samples on scale of 1, good; 2, acceptable; 3, poor. The panel consisted of twelve trained members from our Institute. Each member was asked to repeat the evaluation three times at 1 hr intervals. In this sensory test, randomized complete block design was used; the data were analyzed using analysis of variance.

RESULTS & DISCUSSION

Fouling phenomena

The flux decay of UF prefiltration as a function of process time at various feed flow rates, transmembrane pressures and temperatures is shown in Fig. 2. An empirical equation, $J_t/J_1 = - C(t/t_1)$ (Sheppard and Thomas, 1970), was also used to fit the experimental data; the calculated C values are given in Table 2. Flux declines were not severe at all experimental conditions. During UF prefiltration, the applied transmembrane pressures were relatively low. The effect of pressure on fouling was, therefore, not expected to be significant, as discussed by Hiddink and coworkers (1980). Kuo and Cheryan (1983) studied membrane fouling of acid whey in a spiral-wound unit and pointed out that high flow rates could be beneficial if transmembrane pressure was below 350 KPa. In this study high flow velocity did reduce fouling, most likely due to the efficient removal of foulants from the membrane surface

Table 1—UF and RO testing conditions

Process mode	Transmembrane pressure (KPa)	Feed rate[a]	Temperature (°C)	Feed conc (% T.S.)
UF	21—186	0.16–0.51 (m/sec)	20, 60	2.0
RO	1000—5000	7.3 ±0.1 (L/min)	60	1.86–17.6

[a] The feed rate in UF (hollow fiber unit) is expressed as linear velocity (m/sec) and the rate for RO (plate-and-frame unit) is shown by volumetric flow rate (L/min) due to the difficulty in calculating its linear velocity.

Table 2—C values derived from equation $J_t/J_1 = - C(t/t_1)^a$ for hollow fiber UF prefiltration processes of mushroom blanch water

Flow velocity (m/sec)	Transmembrane pressure (KPa)	Temp (°C)	C values
0.16	82.74	20	0.463×10^{-3}
0.16	124.11	20	0.611×10^{-3}
0.37	62.06	20	0.244×10^{-3}
0.37	103.43	20	0.140×10^{-3}
0.16	124.11	60	1.077×10^{-3}

[a] J_t = flux at time t; J_1 = initial flux, was measured at approximately 1 min after start-on; t_1 = 1 min.

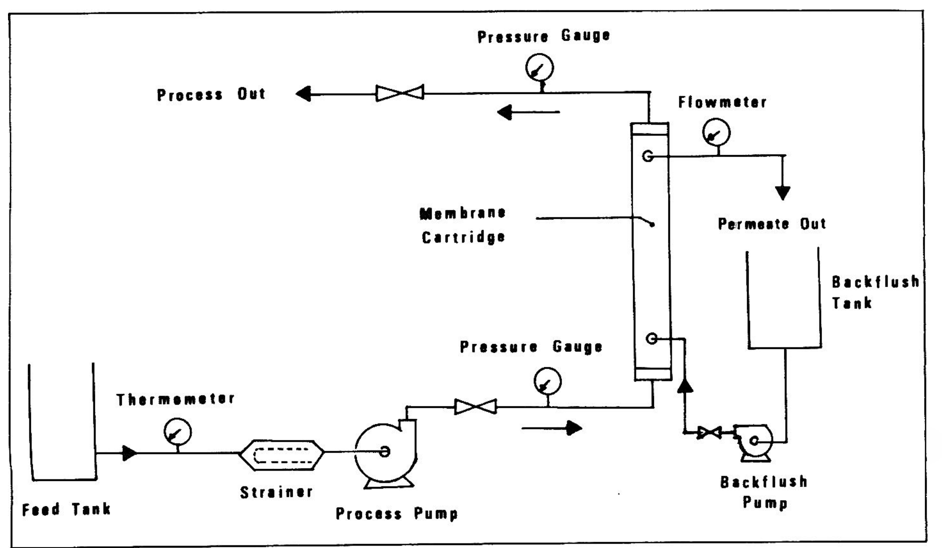

Fig. 1—Process schematic for UF system.

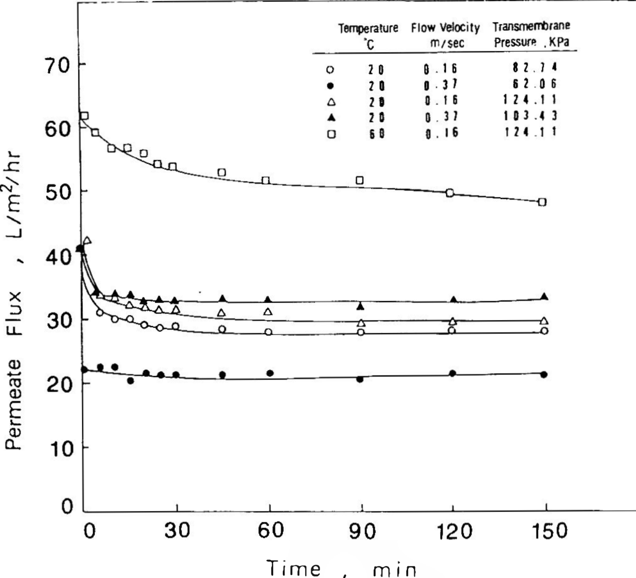

Fig. 2—Flux decay in ultrafiltration of mushroom blanch water.

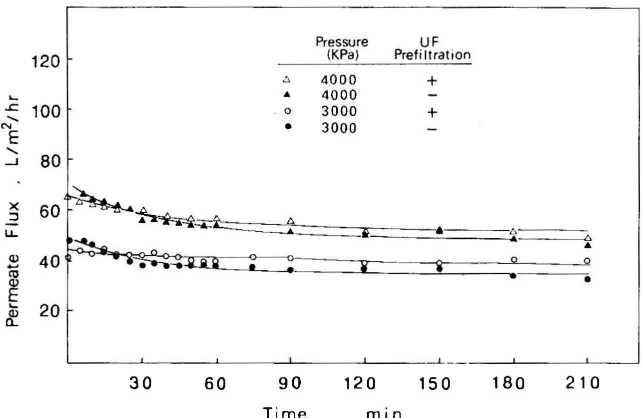

Fig. 3—Flux decay in RO processing of mushroom blanch water of 1.86% solids at 27°C.

at high flow velocity or rather high shear stress. In the case of high temperature operation (60°C), the flux decline in 2½ hr seemed to be slightly greater than that at low temperature. However, higher temperature still maintained much higher flux during processing. Figure 3 and 4 show the fouling phenomena of RO at various operational conditions. At low temperature (27°C), UF prefiltration increased the permeation rate slightly and did not significantly affect fouling rate. However, when RO was operated at higher temperature (60°C), UF prefiltration became absolutely essential. As shown in Fig. 4, without prior UF, the permeate flux dropped rapidly and reached a point even below the permeation rate at low operational temperature within 2 hr. There is no good explanation for this observation at present. Manbois (1980) observed greater fouling rates at high temperature with milk and attributed this fact to the decreased solubility of calcium salts in milk at elevated temperature. Physical properties, such as solubility, of certain components in mushroom blanch water may have been changed by the combination effects of high temperature and pressure during RO processes. In addition, these components are large enough to be rejected by the UF membrane. The mushroom blanch water discharged from the processing line of mushroom canning plants is normally at high temperature and to avoid the severe flux decay during RO, UF prefiltration is indeed a necessary step in mushroom blanch water concentration. To

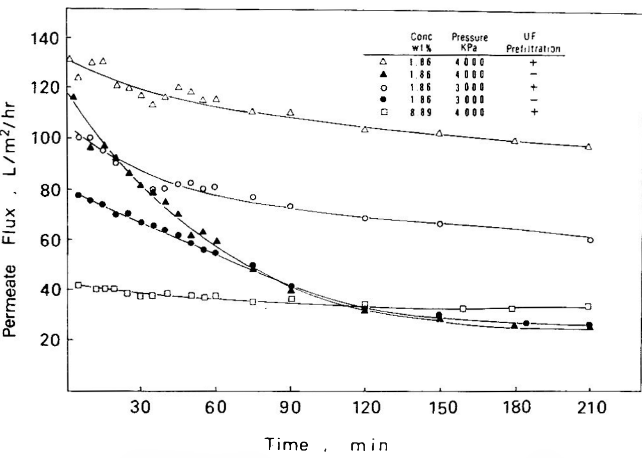

Fig. 4—Flux decay in RO processing of mushroom blanch water at 60°C.

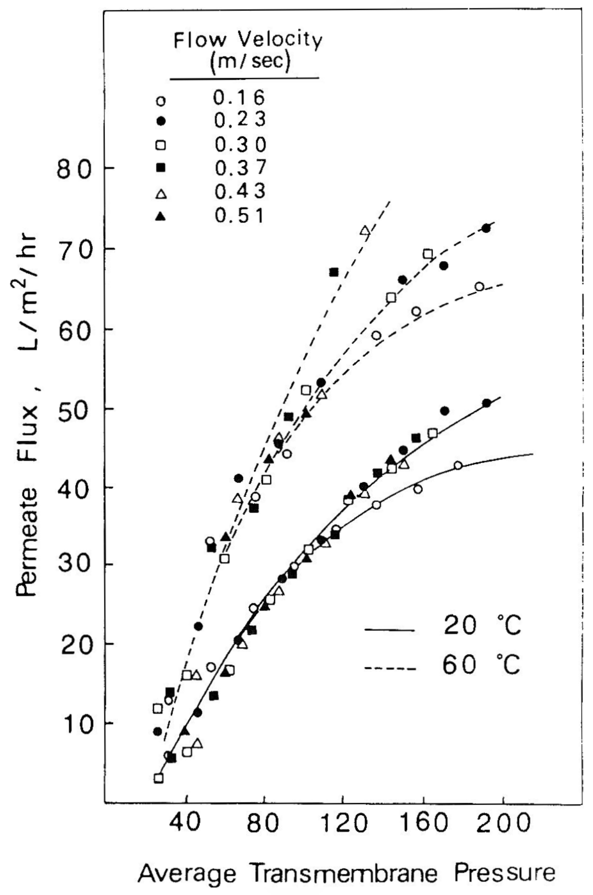

Fig. 5—Relationships between transmembrane pressure and flux of UF prefiltration processes at various flow velocities and temperatures.

investigate if feed concentration would affect RO fouling rate at high temperature and pressure, the UF prefiltered feed was first concentrated (T.S. = 8.89%) and then tested for flux decay at 4000 KPa and 60°C. The result (Fig. 4) showed that after UF prefiltration, the fouling rate of the RO process for high concentration feed was similar to that of the unconcentrated feed, i.e., the rate of fouling was significantly decreased by the UF prefiltration process even when RO was operated at high feed concentration.

Performance of UF and RO processes

The effects of transmembrane pressure and temperature on permeation rate of ultrafiltration are shown in Fig. 5. Both

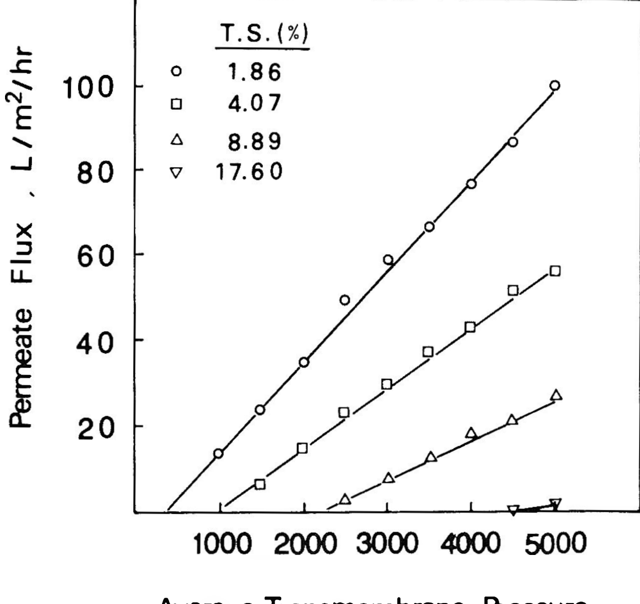

Fig. 6—Effect of feed concentration and transmembrane pressure on permeate flux in reverse osmosis concentration of mushroom blanch water.

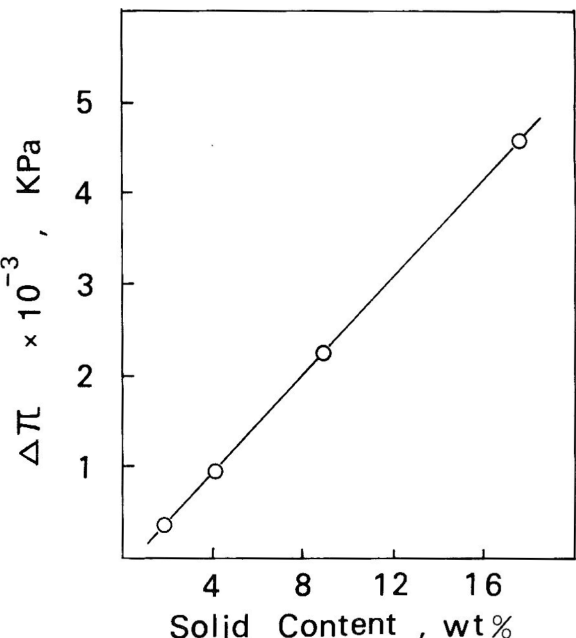

Fig. 7—Variation of flux with feed concentration at 5000 KPa operation pressure during RO process.

temperature and transmembrane pressure exerted significantly beneficial effects on permeate flux. The ultrafiltration process can often be described by:

$$J = \frac{\Delta P_T}{R_m + R_c} \tag{1}$$

where J is the permeate flux; ΔP_T is the transmembrane pressure; R_m is the resistance due to membrane; and R_c is the resistance due to cake formation on the surface of the membrane. Increasing transmembrane pressure would increase driving force and thus increase flux. Increasing temperature not only increased permeability of the membrane (decreased R_m) but also increased back diffusion of the solute from the surface of the membrane (decreased R_c). Since the resistances were reduced at high temperature, flux was expected to be increased as shown by our result. Mushroom blanch water discharged from the processing line is usually at high temperature. Since our results showed that this thermal energy can be used to increase permeation rate in UF prefiltration, high temperature UF prefiltration is recommended.

High flow velocity or rather high shear stress would decrease the thickness of the stagnant boundary layer on the surface of the membrane. Based on the concentration polarization model (Michaels, 1968),

$$J = \frac{D}{\delta}\ln\frac{C_w}{C_b} \tag{2}$$

where J = permeate flux; D = solute diffusivity; δ = thickness of stagnant boundary layer; C_w = solute concentration at wall; C_b = bulk concentration. Decreasing δ would increase the value of D/δ, the mass transfer coefficient and thus increase flux. Possibly due to the low macromolecule concentration in the mushroom blanch water, the phenomenon of concentration polarization was not very significant in our UF processes (Fig. 5). Therefore, at high flow velocity, its effect on the permeation rate was not clear.

In the case of RO concentration, Fig. 6 illustrates the permeation rates of the RO process for ultrafiltered mushroom blanch water at various feed concentrations and transmembrane pressures. Linear relationships between flux and pressures were found at all concentrations. The permeation rate of RO processes

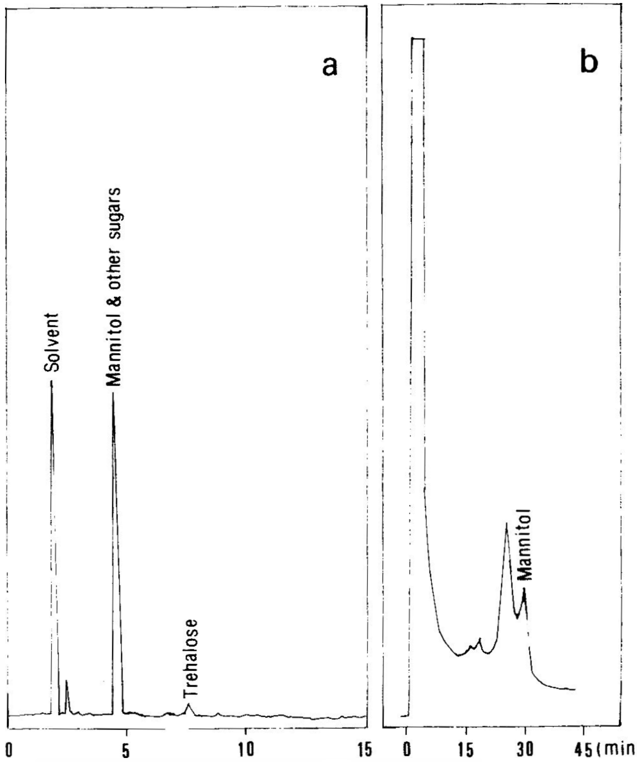

Fig. 8—HPLC profile for sugars of UF permeate of mushroom blanch water (a) and the GC chromatogram of the major peak of HPLC analysis (b).

can be expressed by

$$J = A(\Delta P_T - \Delta \pi) \tag{3}$$

where ΔP_T is transmembrane pressure, $\Delta\pi$ is osmotic pressure and A is the permeability coefficient. The A values and osmotic pressures obtained from our data to fit Eq. (3) are shown in Table 3. The A values of Eq. (3) were found to decrease as the concentration increased, in agreement with the observation made by Merson and Morgan (1968) in RO concentration of juices. For a membrane module system, the A values are affected by the physical properties of feed solution

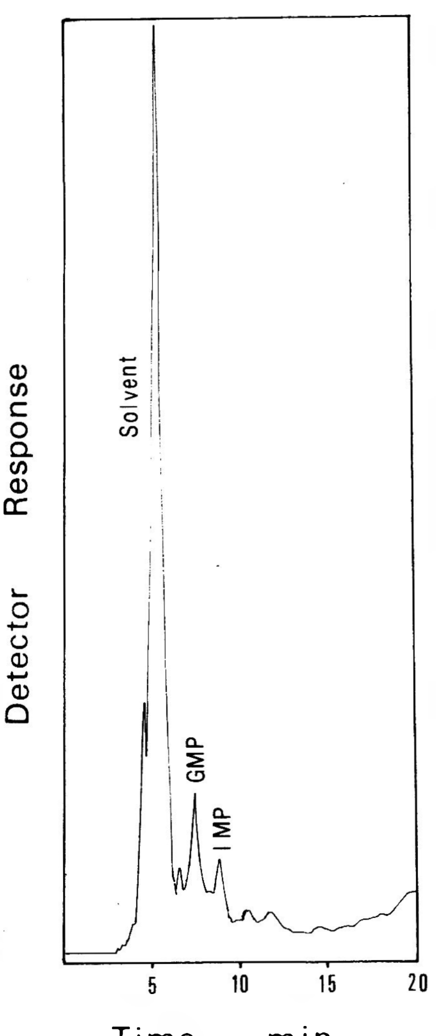

Fig. 9—HPLC profile for nucleotides of UF permeate of mushroom blanch water.

Feed: T.S. 1.97; C.P. 0.802; Ash 0.31; Sug 0.884; IMP 0.0060; GMP 0.0104

UF

UF Concentrate at VCR = 9: T.S. 3.00; C.P. 1.32; Ash 0.41; Sug 0.874; IMP 0.00585; GMP 0.0106

UF Permeate: T.S. 1.86; C.P. 0.718; Ash 0.284; Sug 0.842; IMP 0.00605; GMP 0.0118

RO

RO Concentrate: T.S. 20.30; C.P. 7.90; Ash 3.69; Sug 9.820; IMP 0.0564; GMP 0.123

RO Permeate: T.S. 0.129; C.P. 0.085; Ash 0.0096; Sug 0.039; IMP 0.00033; GMP 0.00072

Fig. 10—Changes of nonvolatile compositions during UF/RO processes of mushroom blanch water. T.S. = total solids; C.P. = crude protein; Sug = major sugars. All the data are expressed as wt% on wet basis.

Table 3—A values and osmotic pressures (Δπ) derived from equation J = A (Δ P_T − Δπ) for plate-and-frame RO concentration processes of mushroom blanch water

Conc (wt.%)	A ($L/m^2/hr/KPa$)	Δπ (KPa)
1.86	0.0211	350
4.07	0.0142	950
8.89	0.0094	2230
17.60	0.0066	4700

Table 4—Percentage recovery of major nonvolatile components in mushroom blanch water when concentrated from 1.97% to 20.3% total solids by UF/RO processes

Total solids	Total nitrogen	Ash	IMP	GMP	Major sugars
95.8	93.0	100	84.2	92.4	90.8

and operation conditions. In this experiment, the operation conditions were fixed. The decrease of A values was most likely due to the increased viscosity, density and decreased diffusivity at high concentration. The osmotic pressures, on the other hand, showed linear relationship with total solids (wt %) of feed, which can be expressed as Δπ = (261.15 KPa) × (wt% total solids). This linear relationship is due to the combination effect of the lack of macromolecules in the feed and good retentivity of the HR-98 membrane of small molecules, which are the major contributors of osmotic pressure in our system.

The relationship between flux and concentration at 5000 KPa is shown in Fig. 7. The concave shape of the curves was due to the decreased A value (increased resistance) at high concentration as discussed previously. The low temperature process would further increase resistances, thus decreasing flux significantly.

Recovery of nonvolatile components in mushroom blanch water by UF and RO

Typical HPLC profiles of sugars and the GC chromatogram of the major peak eluted from HPLC are given in Fig. 8a and 8b. It was found that the major peak in the HPLC chromatogram was actually a mixture of several sugars. Mannitol was identified in this sugar mixture by GC coinjection with standard but there was uncertainty about the other sugars. Hammond and Nichols (1975) reported that the major soluble carbohydrates in fresh mushroom (Agaricus bisporus) were mannitol and trehalose; glucose was present as a minor constituent. Wu and coworkers (1981) reported that glucose was the major sugar in mushroom (Agricus bisporus) blanch water. However, glucose could not be detected either by GC or by the enzymatic method (Bergmeyer and Bernt, 1974) in the present study. Trehalose, however, was found in the blanch water in minor amount as shown in Fig. 8a. The typical HPLC profile of nucleotides is given in Fig. 9. Two major nucleotides, IMP and GMP, were found in this study.

The changes in concentration of nonvolatile components throughout UF/RO processes are shown in Fig. 10. The concentration of major sugars was calculated as mannitol. Total recoveries of nonvolatile components by this processing procedure are given in Table 4. Nucleotides are very potent food flavoring ingredients and may, at least partially, replace the use of MSG (monosodium glutamate) in foods. Our results showed that more than 84% of the major nucleotides were recovered by UF/RO processes. Recoveries of major sugars by UF/RO processes also exceeded 90% in this study. Sugars

Table 5—Percentage recovery of volatile compounds in mushroom blanch water by UF/RO concentration at 60°C

Components	1-octen-3-ol	3-octanol	n-octanol	1-octen-3-yl-propionate	benzyl alcohol
Recovery(%)	53.8	24.6	40.5	70.9	50.0

were the major components, quantitatively speaking, in our final concentrated product (Fig. 10). Recoveries of ash, total nitrogen and other components were also satisfactory (Table 4), demonstrating that UF/RO can recover high percentages of the nonvolatile flavor compounds from mushroom blanch water.

Recovery of volatile components in mushroom blanching water by UF/RO

Major volatile compounds of raw mushroom have been reported to be 1-octen-3-ol, 3-octanol, 3-octanone, n-octanol, benzaldehyde and benzyl alcohol (Cronin and Ward, 1971; Picardi and Issenberg, 1973). Besides the above constituents, 1-octen-3-one can be detected after mushrooms have been boiled for more than 15 min (Picardi and Issenberg, 1973). Typical GC profile of mushroom blanch water is illustrated in Fig. 11. Table 5 gives the percentage recovery of some volatile compounds in the blanch water by UF/RO concentration. It should be noted that vaporization loss during UF/RO concentration processes at 60°C, inevitably, had aggravated the volatiles recovery. The 1-octen-3-ol, known as "mushroom alcohol," provides typical mushroom aroma in the raw mushrooms. Its threshold was reported to be 0.01 ppm (Pyysalo and Shuihko, 1976). Nearly 60% of 1-octen-3-ol was recovered by UF/RO concentration. Due to its low threshold, the 60% recovery of 1-octen-3-ol can still provide the concentrated product with satisfactory mushroom flavor.

To further test the aroma quality of the concentrated product, sensory panels were also used to evaluate the original mushroom blanch water and the reconstituted sample at the same concentration. The average score for original mushroom blanch water was 1.85 and the score for reconstituted sample was 1.96. Statistical analysis showed that there was no significant difference between these two samples ($P > 0.05$). In addition, the differences in scores among panelists were also not significant ($P > 0.05$). Thus, although there was some loss of volatiles during UF/RO concentration processes, the concentrations of the remaining volatiles were still above their thresholds, and the aroma quality changes due to UF/RO concentration were not detectable by sensory tests.

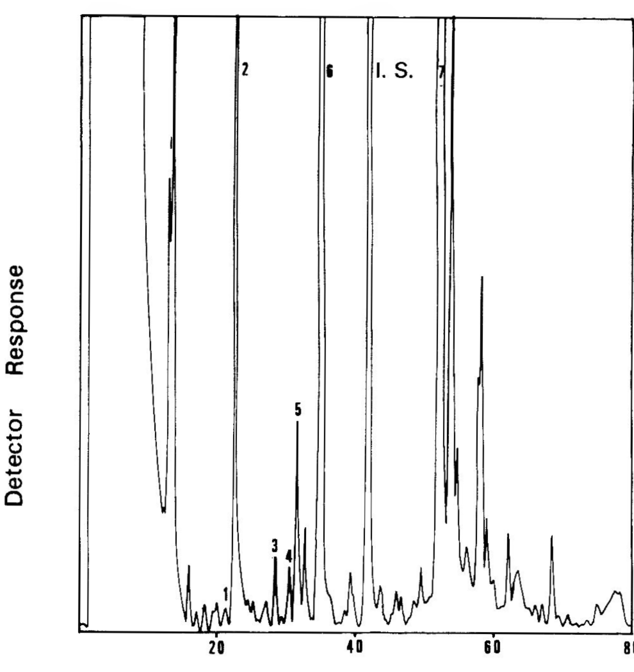

Fig. 11—GC chromatogram of UF permeate of mushroom blanch water, (1) 3-octanone; (2) 1-octen-3-one; (3) 3-octanol; (4) 1-octen-3-ol; (5) 1-octen-3-yl propionate; (6) n-octanol; (7) benzyl alcohol; I. S. (internal standard), n-nonanol.

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Minimum Cooking Time for Potato Strip Frying

C. I. PRAVISANI and A. CALVELO

ABSTRACT

The maximum force for shearing was used as texture parameter for raw and cooked potato strips. The force was satisfactorily correlated with the cook value C (equivalent cooking time at 100°C) showing complete cooking for C ≥ 300 sec. Thermal histories measured at the center of strips fried under different oil temperatures suggested the heat and mass transfer mechanisms involved in the frying process and allowed the calculation of the minimum frying time to assure cooking at the strip center. This time was independent of the oil temperature and slightly dependent on the strip size.

INTRODUCTION

FROZEN FRENCH FRIES represent one of the most important items in the frozen food market. However, the technology for its production requires a permanent adjustment according to the raw material characteristics and the interrelations among the different sequential operations involved. Thus, cold storage of potatoes is followed by blanching, frying and freezing processes, which are completed by the frozen storage and final oil frying or oven reheating.

It is known that the cold storage of potatoes causes an increase in reducing sugar content (Burton, 1969; Iritani and Weller, 1976, 1977; Samotus et al., 1974) that can sometimes be reverted by increasing the temperature during final storage. However, such a procedure involves simultaneously a higher possibility of sprouting (Añón, 1984; Marquez and Añón, 1985). Consequently, in some cases, blanching conditions are adjusted to properly reduce the sugar content (Smith, 1975; Califano and Calvelo, 1983) and to assure a satisfactory color in the frying process. French fries color is the result of a Maillard reaction that depends on the superficial reducing sugar content and the temperature and time of frying (Marquez and Añón, 1985). Thus, the cold storage of raw materials, the blanching and the frying of potatoes are interrelated through the sugar content which affects the final color.

The frying conditions of potato strips must be adjusted to assure cooking at their center, to attain a satisfactory superficial color (depending on the reducing sugar content) and to obtain a proper texture, mainly depending on the thickness and characteristics of the formed crust (Ross and Porter, 1968, 1971). These adjustments of operating conditions which should take into account the raw potato characteristics as well as the cool storage conditions require a quantification of the different effects to predict and optimize results.

There are few papers on the mathematical modeling of potato frying (Beloborodov and Konavalov, 1977; Ashkenazi et al., 1984) and in general, there is little agreement on the heat and mass transfer mechanisms involved in the process.

In this study texture of potatoes cooked under negligible temperature gradients was measured to determine the ideal cooking point in terms of an equivalent cooking time. In addition temperatures at the center of potato strips were measured under different frying conditions. From this information a cooking model for the processing of French fries was outlined and the minimum time to assure cooking inside the potato strips during frying was calculated.

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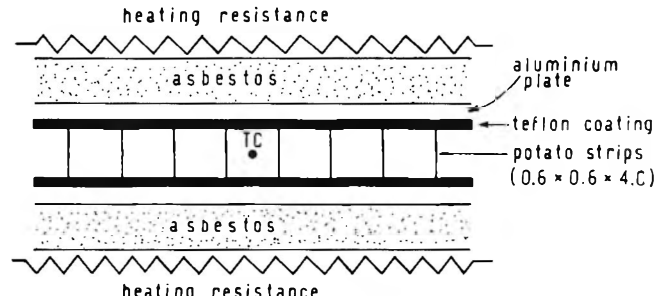

Fig. 1—Schematic drawing of the strip cooking device.

MATERIALS AND METHODS

Samples

Tubers of Kennebec cultivar were stored at 4°C in darkness up to 24 hr before runs. Potatoes of similar sizes (10 cm long and 7 cm diameter) were selected for the tests.

Tubers were cut along the axis forming strips of either 0.6 × 0.6 × 4.0 cm or 1.0 × 1.0 × 5.0 cm according to the scheduled test. The strips were classified into three kinds depending on the concentric layer to which they belonged (Ross and Porter, 1971). Texture tests were performed on samples of each different layer and on samples including fractions of each layer according to the proportion existing in the potato. The same procedure was adopted for cooked potatoes except that two groups of strips were formed: one was cooked and the other was used as a reference.

Cooking

The cooking of strips was accomplished under such conditions that the absence of temperature gradients inside the samples was assured. The cooking equipment consisted of two electrical heating plates between which the sample was placed. Teflon coated aluminum plates were placed between the sample and the heating plates to avoid potato sticking and to provide homogeneous temperature distribution.

Asbestos plates were also added to control the temperature drop so that the sample would increase in temperature without internal gradients. A schematic of the cooking device is shown in Fig. 1. The heating plates were connected to two auto-transformers independently controlled, which allowed heat control and produced different thermal treatments.

The temperature of the sample was measured by means of copper-constantan thermocouples placed at the center and on the surfaces of the aluminum plates. Melting ice was used as reference and temperatures were measured by connecting the thermocouples to a multichannel potentiometric recorder. From the temperatures, the cook value C or cooking time equivalent at 100°C, was calculated as defined by Leonard et al. (1964):

$$C = \int_{T_i}^{T_e} 10^{(T - T_r)/Z_c} dt \qquad (1)$$

where T_i is the initial temperature; T_e the final temperature; T_r = 100°C, the reference temperature; and Z_c is the necessary temperature rise for a 10-fold increase in reaction rate for chemical, physical or sensory changes. Z_c = 17°C was used according to the value reported by Dagerskog (1977) for the rheology of potatoes.

Table 1—Texture measurements in concentric layers of raw potatoes

Maximum shear force (kg) — 2 strips per run			
	Concentric layers		
Tuber	Outer	Intermediate	Inner
1	27.3 (8)[a]	24.7 (7)	28.4 (4)
2	37.0 (8)	31.7 (9)	31.0 (6)
3	40.2 (8)	38.8 (7)	38.6 (8)
4	42.2 (8)	38.2 (8)	38.6 (4)

Source	SS	ø	s^2	F
Tuber	2309.50	3	769.83	47.46**
Zone	180.46	2	90.23	5.56**
Error	1281.21	79	16.22	

[a] Number of runs averaged shown in parentheses.

Table 2—Texture measurements in representative groups of the different concentric layers

Maximum shear force (kg) — 2 strips per run			
	Groups		
Tuber	1	2	3
1	34.8 (6)[a]	31.5 (7)	33.4 (6)
2	32.6 (12)	32.0 (14)	32.9 (12)
3	40.6 (8)	38.4 (7)	38.6 (8)
4	40.8 (6)	38.6 (7)	40.4 (7)
5	25.0 (7)	26.8 (6)	28.1 (6)
6	33.0 (8)	33.9 (7)	33.3 (8)

Source	SS	ø	s^2	F
Tuber	2501.05	5	500.21	32.39**
Group	25.41	2	12.70	1.22
Error	2068.78	134	15.44	

[a] Number of runs averaged shown in parentheses.

Texture

An Instron Universal Tester with a Kramer shear cell as sensor element was used. This multiple blade device made the sample undergo combined shear and normal stresses and by working with more than one strip, it was possible to obtain representative results of the whole potato texture. The advance rate of the ram was 100 mm/min in all cases. The maximum shear force, F, was taken as the measured value. Experiments were performed on 0.6 × 0.6 × 4.0 cm strips.

Frying

The frying of potato strips was carried out in a thermostatic oil bath controlled at ± 0.2°C. The bath was specially designed with an important exchange area and was agitated by means of a circulating pump. The bath volume was 25L which assured a minimum change of temperature when immersing the samples. Oil temperatures ranging from 150 to 200°C were used. The temperatures of the samples were measured by copper-constantan thermocouples placed at their centers and connected to a multichannel potentiometric recorder. Thermocouple wire diameters were 0.6 mm. A reduction of the wire diameters to 0.06 mm did not appreciably change the results. The size of the strips was 1.0 × 1.0 × 5.0 cm.

Water content

The water content was measured on potatoes according to the AOAC (1970) method.

RESULTS & DISCUSSION

Raw potato texture

Texture tests on groups of two strips of raw potatoes showed significant differences among the concentric layers as well as among potatoes. Results are shown in Table 1 together with the corresponding analysis of variance. Table 2 shows texture values of samples consisting of strips of different layers in the same proportion that exists in the potato (groups). Tests were run on two strips at a time, and the reported average values corresponded to no less than six experiments. The analysis of variance (Table 2) shows that there is a significant difference among potatoes though not among groups; therefore, it was possible to use one of them as a raw control while the other ones were cooked.

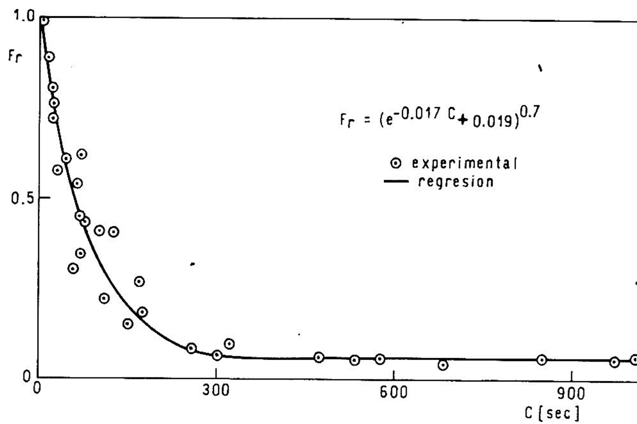

Fig. 2—Normalized shear force, F_r *as a function of cook value C.*

Cooked potato texture

The results obtained for raw potatoes led to the following sample method: the strips obtained from a potato were divided into two or more representative fractions of the tuber (groups). One of the groups was tested in raw state. The remaining groups were cooked under controlled conditions and then tested for texture. Tests were run on two strips at a time and results of the group were averaged. (Each group included 6 to 14 tests). This method did not avoid the differences in texture among potatoes; therefore, to compare results in cooked tubers, each value was referred to that of the corresponding raw potato (Brown and Morales, 1970). Thus, a normalized force for shearing, F_r, was defined as:

$$F_r = F_c/F_k \quad (2)$$

where F_c is the maximum force for shearing of the cooked potato and F_k is the corresponding value of the raw tuber.

Figure 2 shows the normalized force, F_r, as a function of the cook value C. It can be noticed that in terms of the normalized force, the differences among potatoes have almost disappeared even with values of F_k ranging from 31 to 47 kg. Figure 2 also shows a sudden decrease of texture during the early stages of cooking, and a tendency towards an asymptotic value at $F_r = 0.05$ for long periods of time, even under overcooking conditions. Similar results were reported by Celba (1979).

Results were analyzed by an adjustment method for nonlinear equations (Marquardt, 1963). Data were properly represented by the equation:

$$F_r = [a + \exp(-bC)]^d \quad (3)$$

where a = 0.019; b = 0.017 sec^{-1} and d = 0.7.

By direct observation (transparency) as well as by taste, samples could be considered cooked when they reached a value of $F_r = 0.05$. Figure 2 shows that this value is in agreement with the beginning of the asymptotic zone which allowed one to determine objectively the minimum cooking time as C = 300 sec.

Application to potato strip frying

During frying, potato strips showed important temperature gradients which indicated different local temperatures and a profile of C values. To apply the texture results summarized by Eq. (3) to the frying of potato strips, the temperatures at the center (the most difficult point to cook) for different temperatures of the oil bath must be known. Once the center is cooked, the rest of the strip which surely attains C values

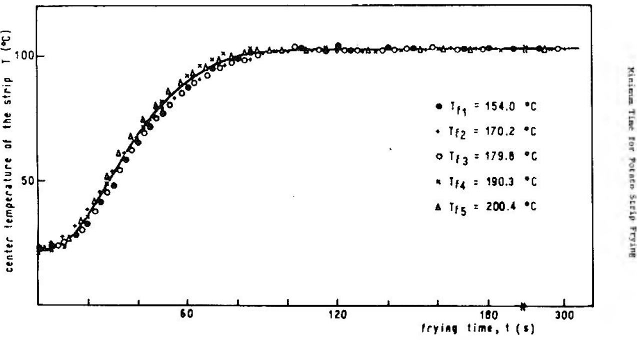

Fig. 3—Thermal histories of the potato strip center for different oil bath temperatures.

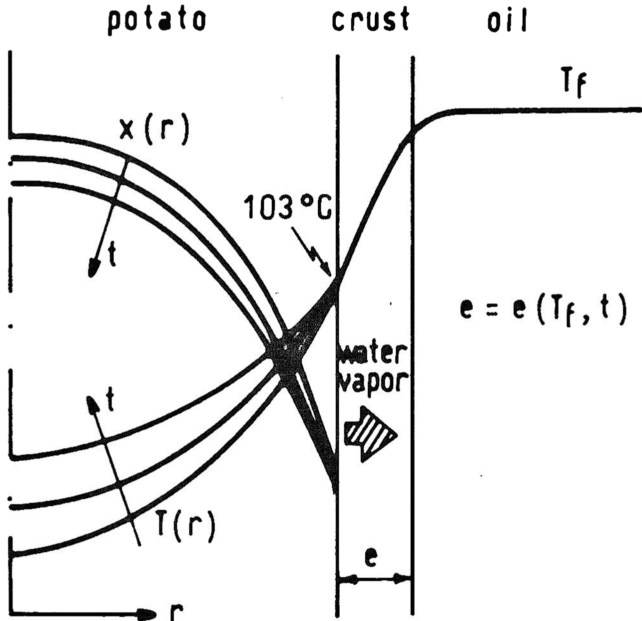

Fig. 4—Scheme of the heat and mass transfer mechanisms involved in potato strip frying.

higher than 300 sec, will also be cooked with $F_r = 0.05$. This value will not be the total texture of the fried strip since it also involves an important contribution of the thin dehydrated crust formed on the surface which was not considered in this study (Ross and Porter, 1968). However, from the results it was possible to estimate the minimum processing time to assure cooking at the center of the strip. Experiments on potato strip frying for different temperatures of the oil bath were performed by measuring the temperature at the center. Results (Fig. 3) show that for the wide range assayed (154–200°C), the oil bath temperature does not affect the temperature at the center of the strips. Even for oil temperatures as high as 200°C, the center temperature tends toward an asymptotic value of 103°C. At the same time, experiments carried out with thermocouples placed at different positions in the strip showed the existence of important gradients although for long periods, temperatures also tended towards 103°C. The tendency of the temperature inside the strip toward an asymptotic value much lower than and independent of the oil bath temperature suggested the existence of an evaporating moving boundary that produced a prescribed temperature condition (103°C) at some point near the strip surface. Figure 4 was sketched on this basis and proposes the mechanisms of heat and mass transfer involved during strip frying. The figure shows a moving boundary in which water evaporation takes place. This boundary defines the limit between cooked potato and crust and is maintained at 103°C

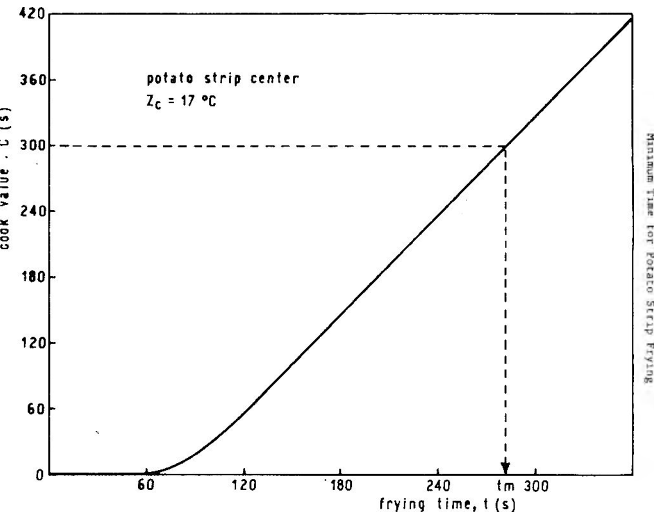

Fig. 5—Cook value at the potato strip center as a function of frying time.

(superheated) as a requirement for nucleation of steam bubbles (Califano and Calvelo, 1985). The liquid water would move from inside the strip toward the boundary, where it would be evaporated and transferred through the crust as vapor. Higher temperatures of the oil bath would increase the boundary rate of advance but would maintain the same prescribed temperature on the boundary (T_o = 103°C). Since the crust thickness was about 0.5 mm, the described pattern led to temperatures within the potato independent of the oil temperature and tending to a final temperature of 103°C. Likewise, the water flux arriving at the boundary from inside the strip, could also be nearly independent of the oil temperature (Fig. 4). In other words, at equal times, higher oil temperatures would produce larger crust thickness though with the same dehydration in the inner part of the strip.

By introducing the experimental temperatures of Fig. 3 into Eq. (1), the cook value at the center of the strip as a function of time could be calculated. Results, shown in Fig. 5, allow the estimation of the minimum frying time needed to assure cooking at the center of the strip. Thus, if the cook value obtained from texture studies (C = 300 sec) is used, Fig. 5 shows that frying time cannot be shorter than 282 sec. This result is independent of the oil temperature used.

On the other hand, inspection of Fig. 3 shows that the time needed to reach the maximum temperature (103°C) at the center of the strip is only 25% of that needed for cooking. The reduction of strip thickness would only shorten that 25% of the total time; therefore, an important reduction of the frying time by reducing the strip sizes should not be expected.

CONCLUSIONS

USING THE KRAMER CELL and taking representative samples of the different concentric layers, reproducible values of the maximum shear force can be obtained for samples of the same potato.

A satisfactory correlation with the cook values, independent of the raw potato texture, can be obtained by normalizing the maximum force for cooked potatoes by means of the corresponding value for the raw sample. The potato can be considered cooked when it reaches a cook value C = 300 sec that corresponds to a reduction of the maximum force to 5% of the initial value. The overcooking (up to 1000 sec) does not involve an additional reduction in texture.

From the temperatures measured at the center of strips fried under different oil temperatures, the heat transfer mechanism involved in the frying process can be outlined. Thus, it is proposed that the existence of a boundary at 103°C that sep-

arates potato and crust explains the independence of the temperatures at the strip center from the oil temperature.

The minimum frying time to assure the cooking of the strip could be estimated as $t_m = 282$ sec from the calculation of the cook value C at the strip center. This time was independent of the oil temperature and did not change very much when strip sizes were reduced. Higher oil temperatures for a given time, led to larger crust thickness; therefore, the frying time and temperature for the strips will depend on color and texture requirements though it has to comply with the minimum time, t_m, to assure the cooking of the strip center.

NOMENCLATURE

a	= Parameter defined in Eq. (3)
b	= Parameter defined in Eq. (3) (sec^{-1})
C	= Cook value (sec)
d	= Parameter defined in Eq. (3)
e	= Crust thickness (m)
F_c	= Maximum shear force for cooked potatoes (kg)
F_k	= Maximum shear force for raw potatoes (kg)
F_r	= Normalized maximum shear force, $F_r = F_c/F_k$
r	= Transversal coordinate (m)
t	= Time (sec)
t_m	= Minimum frying time (sec)
T	= Temperature (°C)
T_f	= Oil bath, temperature (°C)
T_e	= Final cooking temperature (°C)
T_i	= Initial cooking temperature (°C)
T_r	= Reference temperature; T_r = 100°C
X	= Water content dry basis
Z_c	= Parameter defined in Eq. (1) (°C)

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Modeling of Sulfur Dioxide Uptake in Pre-Peeled Potatoes of Different Geometrical Shapes

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ABSTRACT

Sulfur dioxide uptake by pre-peeled potatoes from dipping solutions was mathematically modeled. Diffusive mass transfer equations in porous medium were experimentally verified; residual levels of sulfur dioxide were measured in the range of industrial operating conditions. Effects of sodium bisulfite solution concentration, immersion time, size, shape, dry matter, density and velocity of the product were analyzed. Three geometric approximations to pre-peeled potato cuts were examined (spheres, cubes and parallelepipeds). The fitting of equations to experimental data determined the effective diffusion coefficient of sulfur dioxide in potato tissue, which was compared to theoretical predictions in terms of molecular diffusivity, total solids content and tortuosity factor.

INTRODUCTION

DISCOLORATION of peeled or cut raw potatoes is a result of enzymatic oxidation of injured cells exposed to oxygen. This type of discoloration is of commercial importance in the potato pre-peeling industry. Blackening of raw tubers is due to enzymatic oxidation of tyrosine and other o-dihydric phenols to melanins. The major enzyme system responsible for the discoloration of potatoes after injury is generally accepted to be polyphenol oxidase. Chlorogenic acid also is involved, at least in part, in the enzymatic browning of injured potato tissue.

Considerable work has been published on practical methods for preventing the discoloration of peeled potatoes. Most of these investigations have been reviewed by Feinberg et al. (1975) and Smith (1977). The use of salts of sulfur dioxide to control discoloration in pre-peeled potatoes is well established in commercial practice (Nieuwenhuis and Van Nielen, 1974; Keijbets, 1981); one of the first patents was issued to Draper (1934).

Numerous attempts have been made to find a substitute for treating with sulfite (Olson and Treadway, 1949; Anderson and Zapsalis, 1957; Amla and Francis, 1959). The different agents described in trade literature have not been used commercially because of high cost and some undesirable side effects such as leakage and off-flavors. Sulfur dioxide has the advantage of being somewhat antiseptic in addition to its function as an inhibitor of discoloration. Sulfur dioxide and sulfite, bisulfite or metabisulfite salts set up a pH dependent equilibrium mixture when dissolved in water. As the pH falls, the proportion of SO_2 increases; this is important in connection with the antimicrobial activity of sulfur dioxide that is largely related to the unbound nonionized molecular form (Clark and Taka'cs, 1980).

Excessive treatment with sulfur dioxide causes abnormal appearance of the product, undesirable flavor softening and weeping from potato tissue (Amla and Francis, 1961; Francis and Amla, 1961). There is a marked tendency to decrease the amount of sulfite retained by the product. Vacuum packing has been reported to reduce sulfite requirements with respect to conventional packaging in gas permeable films (Anderson and Zapsalis, 1957). Several factors such as: color preservation, microbial growth, weep production and softening of the tissue must be taken into account to establish the adequate antioxidant treatment in terms of the optimum level of residual sulfur dioxide in pre-peeled potatoes.

Limited information has been available in the literature about the effects of dipping solution concentration, immersion time, size, shape and velocity of the product on the amount of sulfur dioxide retained by potato cuts (Ross and Treadway, 1961; Francis and Amla, 1961; Furlong, 1961).

The purpose of this study was to propose a mathematical model describing the uptake of sulfur dioxide in pre-peeled potatoes of different geometry and size as a function of: (1) the product physical properties, and (2) the industrial operating parameters (concentration of dipping solution and sample rectilinear velocity). The equations were derived from the volumetric integration of the concentration profiles and were tested for different experimental conditions.

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THEORY

POTATO TISSUE was assumed to consist of an insoluble matrix (starch, cellulose, pectic substances) and an aqueous phase through which sulfur dioxide diffuses. Unsteady state diffusion in symmetric porous materials can be analyzed using a general form of the microscopic mass balance:

$$\frac{\partial c}{\partial t} = \frac{1}{r^{\phi-1}} \frac{\partial}{\partial r}\left(r^{\phi-1} D_e \frac{\partial c}{\partial r}\right) \quad (1)$$

where c is the solute concentration in the solid as a whole (weight of sulfur dioxide per unit volume of potato); $\phi = 1$ for an infinite slab, 2 for an infinite cylinder, 3 for a sphere; r is the distance measured from the center of the solid, and D_e is the effective diffusion coefficient of sulfur dioxide in the potato tissue which can be expressed in terms of molecular diffusivity (D_{AB}), porosity (ϵ) and tortuosity factor (Ω) (Sherwood et al., 1975):

$$D_e = \frac{D_{AB}\,\epsilon}{\Omega} \quad (2)$$

The porosity is the volume fraction of solid occupied by the occluded liquid; the tortuosity is the ratio of the diffusion path to the nominal distance traversed by the solute (Schwartzberg and Chao, 1982).

Spheres, cubes and parallelepipeds (French fry strips) are common geometrical shapes in the pre-peeled industry. A detailed description of equations is only shown for spherical geometry; in the case of spheres, Eq. (1) can be written in the form:

$$\frac{\partial c}{\partial t} = \frac{1}{r^2}\left(\frac{\partial}{\partial r} r^2 D_e \frac{\partial c}{\partial r}\right) \quad (3)$$

with the following initial and boundary conditions:

$$t \leq 0 \qquad c = 0 \qquad 0 \leq r \leq R \quad (4)$$

$$t > 0 \quad D_e \frac{\partial c}{\partial r} = \epsilon\, k_L (c'_f - c')$$
$$= k_L (c_f - c) \quad \text{in} \quad r = R \qquad (5)$$
$$t > 0 \quad \frac{\partial c}{\partial r} = 0 \quad \text{in} \quad r = 0 \qquad (6)$$

In Eq. (5), k_L is the mass transfer coefficient at the potato-fluid interface; c'_f, the sulfur dioxide concentration in dipping solution (weight of sulfur dioxide per unit volume of solution); and ϵ, the porosity of the potato tissue (water content on wet basis). The equilibrium distribution ratio between the solute concentration in the liquid bath (c') and the solute concentration in the solid (c) was given by $c = c' \epsilon$ assuming that the partition coefficient equals one.

The solution of Eq. (3) to (6) in the form of a dimensionless concentration profile is given by (Crank, 1957):

$$c^* = 1 - \frac{2\,Bi}{r^*} \sum_{n=1}^{\infty} \frac{e^{-\gamma_n^2 t^*}}{\gamma_n^2 + Bi(Bi - 1)} \frac{\sin(\gamma_n r^*)}{\sin \gamma_n} \qquad (7)$$

where $c^* = c/c_f$; $r^* = r/R$; $t^* = (D_e\, t)/R^2$; Biot number, $Bi = (k_L R)/D_e$; and γ_n, $n = 1, 2 \ldots$ are the roots of:

$$\gamma_n \cot \gamma_n = 1 - Bi \qquad (8)$$

Eq. (7) was integrated over the total sample volume (V) and the fractional uptake (F) was calculated as follows:

$$F = \frac{Mt}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{6\,Bi^2\, e^{-\gamma_n^2 t^*}}{\gamma_n^2 \{\gamma_n^2 + Bi(Bi - 1)\}} \qquad (9)$$

When product velocities through the surrounding fluid corresponded to high Biot number (Bi > 200), constant interfacial concentration was assumed; the integration of concentration profile in this case was given by:

$$\frac{Mt}{M_\infty} = 1 - \frac{6}{\P^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left(-\frac{n^2 \P^2 t^*}{R^2}\right) \qquad (10)$$

In the modeling of sulfur dioxide diffusion in cubes and French fry potato strips (parallelepipeds) concentration profiles for an infinite plane sheet were applied. Three-dimensional contributions have been considered using Newman's Rule (Carslaw and Jaeger, 1959). Dimensionless concentration profiles were integrated over the corresponding sample volume.

Fractional uptake for cubes and potato strips were calculated as follows:

$$F = \frac{Mt}{M\infty} = 1 - \sum_{n=1}^{\infty} \sum_{m=1}^{\infty} \sum_{\ell=1}^{\infty} \frac{8\left(\frac{Bix}{\beta_n}\right)^2 \left(\frac{Biy}{\beta_m}\right)^2 \left(\frac{Biz}{\beta_\ell}\right)^2 \exp\left\{-D_e t \left(\frac{\beta_n^2}{a^2} + \frac{\beta_m^2}{b^2} + \frac{\beta_\ell^2}{d^2}\right)\right\}}{(Bi_x^2 + Bi_x + \beta_n^2)(Bi_y^2 + Bi_y + \beta_m^2)(Bi_z^2 + Bi_z + \beta_\ell^2)} \qquad (11)$$

where a, b, d are the half thickness values of the solid in the x, y, z directions respectively,

$$Bi_x = \frac{k_L\, a}{D_e}; \; Bi_y = \frac{k_L\, b}{D_e}; \; Bi_z = \frac{k_L\, d}{D_e}$$

and

$$\beta_n \tan \beta_n = Bi_x; \; \beta_m \tan \beta_m = Bi_y; \; \beta_\ell \tan \beta_\ell = Bi_z \qquad (12)$$

An average mass transfer coefficient k_L was adopted for x, y, z directions.

In cases of constant interfacial concentration approach the following expression was derived for cubes and potato strips:

$$F = \frac{Mt}{M_\infty} = 1 - \left(\frac{64}{\P^3}\right)^2 \frac{1}{8} \left\{ \sum_{\ell=0}^{\infty} \sum_{m=0}^{\infty} \sum_{n=0}^{\infty} \frac{\exp(-\alpha_{\ell,m,n} t)}{(2\ell+1)^2 (2m+1)^2 (2n+1)^2} \right\}$$

$$\text{where } \alpha_{\ell,m,n} = \frac{\P^2 D}{4} \left\{ \left(\frac{2\ell+1}{a}\right)^2 + \left(\frac{2m+1}{b}\right)^2 + \left(\frac{2n+1}{d}\right)^2 \right\} \qquad (13)$$

In Eq. (9, 10, 11, 13) $M_\infty = c'_f\, \epsilon\, V$, where V is the potato sample volume according to its geometrical shape. Residual sulfur dioxide per unit weight of a potato cut (W) for a given time, was calculated as follows:

$$W\,(\text{ppm}) = \frac{F\, c'_f\, \epsilon}{\rho} \times 10^6 \qquad (14)$$

Mass transfer coefficients in dipping solution were evaluated using the following equations (Sherwood et al., 1975), in terms of Reynolds and Schmidt numbers.

$$\frac{k_L\, 2R}{D_{AB}} = 2 + 0.6\, Re_s^{1/2}\, Sc^{1/3} \; \text{(spheres)} \qquad (15)$$

$$\frac{k_L\, 2L}{D_{AB}} = 0.664\, Re_p^{1/2}\, Sc^{1/3} \; \text{(laminar boundary layer on a flat plate)} \qquad (16)$$

Model equations for each geometry were fitted to experimental data by varying the effective diffusivity D_e to minimize least squared differences and residues sum.

MATERIALS & METHODS

SAMPLES of potatoes (*Solanum Tuberosum*, Kennebec variety from Balcarce, Pcia. de Buenos Aires) with different geometrical shapes and sizes were dipped in solutions of sodium bisulfite, subjected to rectilinear movement at constant velocities through the stationary fluid and analyzed for sulfur dioxide as a function of time.

Percent total solids and specific gravity of the samples were determined according to the methods reported by Porter et al. (1964) and Fitzpatrick et al. (1969). Solids were in the range 20–22% and average density value was 1087 kg/m^3. The potatoes were hand peeled, cut into spheres (radius R = 1.1–2.87 cm), cubes (1 × 1 × 1 cm) and French fry strips (parallelepipeds of 1 × 1 × 5 cm and 1 × 1 × 7 cm). Dip solutions were made by dissolving the appropriate amount of sodium bisulfite in distilled water. Experiments were performed at 20°C with solutions of 0.25, 0.5, 0.8, and 1.7% of sodium bisulfite equivalent to 1538, 3076, 4922, and 10466 ppm of sulfur dioxide in dipping solution, respectively. Rectilinear velocities of potato cuts in the antioxidant bath ranged from 0.010 to 0.030 m/sec; immersion times varied from 30 sec to 2 min.

Residual levels of sulfur dioxide in pre-peeled potatoes were determined simultaneously by two methods: (a) iodometrically (Hart and Fisher, 1971), and (b) colorimetric method (AOAC, 1980).

The γ_n, $\beta_{n,m,\ell}$, roots (Eq. 8 and 12) were determined from a computational algorithm using a modified False Position method. More than thirty terms of the series were calculated in each geometry to get convergence, because of the short immersion times involved in the diffusional process.

Theoretical uptake for different geometries obtained from model equations were compared to experimental data of residual sulfur dioxide to verify the applicability of the diffusive mass transfer model. Percent deviation (%E) was calculated according to:

$$\%\,E = \sqrt{\frac{\sum_{n=1}^{N} \left(\frac{W_t - W_e}{W_e}\right)_n^2}{N - 1}} \times 100 \qquad (17)$$

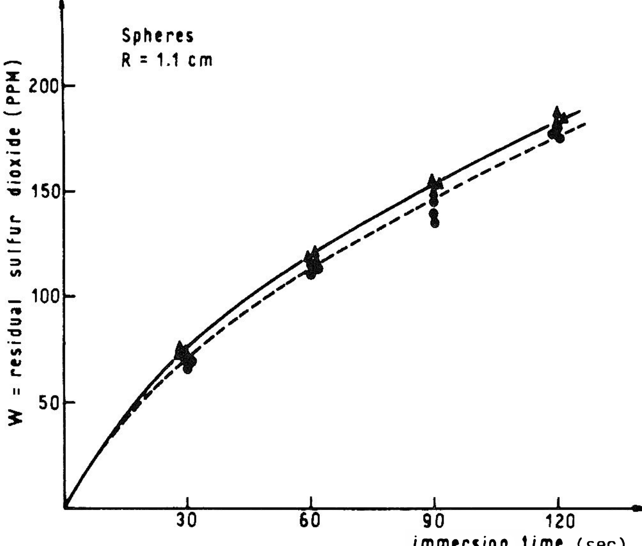

Fig. 1—Comparison of predicted and experimental sulfur dioxide uptake in spherical pre-peeled potatoes. R = 1.1 cm. Dry matter 22%. Concentration of dipping solution 0.5% sodium bisulfite. Experimental ▲ v = 0.028 m/sec; ● v = 0.020 m/sec. Predicted — k_L = 9.75 × 10^{-6}m/sec, Bi = 99.4; --- k_L = 8.24 × 10^{-6}m/sec, Bi = 84.0.

where W_t and W_e represented the theoretical and experimental residual sulfur dioxide content respectively and N the number of experimental data considered.

RESULTS & DISCUSSION

SULFUR DIOXIDE uptake was strongly dependent on dry matter content of the sample. Density and total solids varied along the tuber; different values corresponded to cortical, perimedullary and pith tissues. For samples with an average dry solids matter of 22%, the effective diffusion coefficient which minimized differences between theory and experiment was D_e = 1.078 × 10^{-9} m^2/sec. This value can also be predicted using Eq. (2) and considering that $D_{SO_2 - H_2O}$ = 1.7 × $10^{-9}m^2$/sec at 25°C (Perry and Chilton, 1973), ϵ = 0.78 (total solids content = 22%) and adopting a tortuosity factor Ω = 1.23 similar to that reported by Califano and Calvelo (1983) and obtained by Stahl and Loncin (1979).

Spherical samples of R = 1.1 cm were immersed in solutions of different sulfur dioxide concentration with rectilinear velocities of 0.020 and 0.028 m/sec (similar to industrial conditions) and analyzed for SO_2 residual values. Figure 1 corresponds to a solution concentration of 0.5% sodium bisulfite (equivalent to 3076 SO_2 ppm in the fluid). Theoretical uptake curves were obtained fitting the following values in Eq. (9): D_e = 1.078 × $10^{-9}m^2$/sec, k_L = 9.75 × 10^{-6}m/sec, (Bi = 99.4) in the case of v = 0.028 m/sec, and k_L = 8.24 × 10^{-6}m/sec, (Bi = 84.0) for v = 0.020m/sec. Mass transfer coefficients were calculated using Eq. (15); physical properties of liquid solution have been included in Re and Sc numbers calculations.

A linear relationship between uptake values and dip solution concentration was predicted using Eq. (14) and experimentally verified in spherical geometry for different velocities and immersion times (Fig. 2). This linear relationship was also valid in cases of cubes and parallelepipeds. Similar effects of dipping solution concentration on residual SO_2 in pre-peeled potatoes have been reported (Francis and Amla, 1961; Furlong, 1961; Ross and Treadway, 1961). Percent deviation of the model (Eq. 17) was 1.83% for spherical geometry. Model

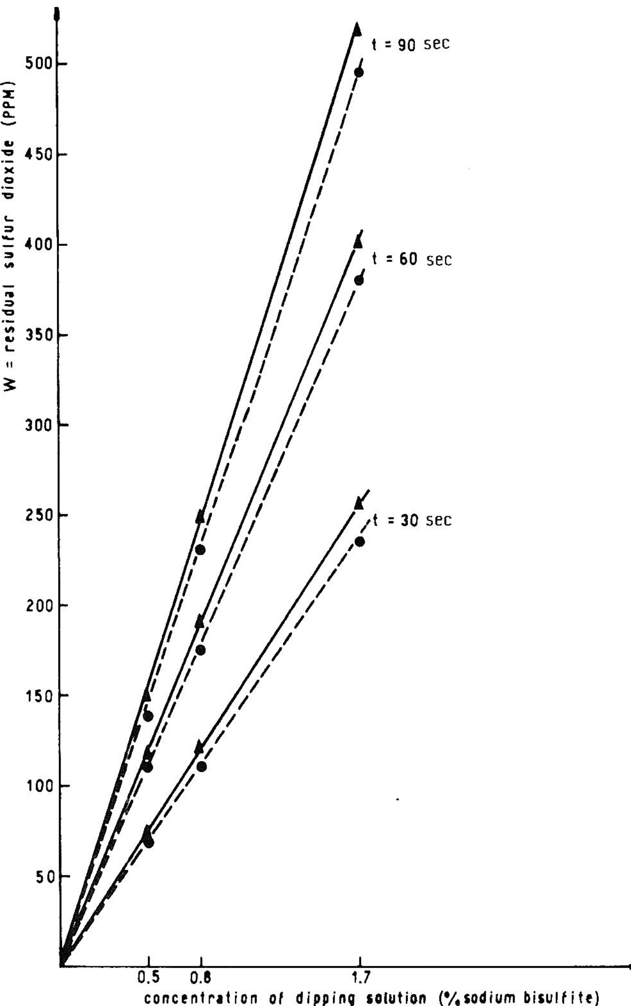

Fig. 2—Influence of dipping solution concentration on sulfur dioxide uptake. Sphere, R = 1.1 cm. Experimental ▲ v = 0.028 m/sec; ● v = 0.020 m/sec. Predicted — k_L = 9.75 × 10^{-6} m/sec; --- k_L = 8.24 × 10^{-6} m/sec.

equations were used to analyze the influence of potato cuts velocities and sizes on residual sulfur dioxide values.

Theoretical curves (Fig. 3) show the effects of Biot number in spheres (R = 1.1 cm) with external concentration of 0.5% sodium bisulfite. When these samples were immersed into a stagnant solution and were motionless, Biot number was 1.5; for sample velocities of 0.028 m/sec, Bi ≅ 100. Mass transfer has a mixed control in the velocity range of industrial operating conditions. Constant interfacial approach was included in the upper curve denoted by CC.

Size of spherical samples has a marked influence on uptake values in relation to whole pre-peeled potatoes. Figure 4 shows this effect for samples with R = 1.1, 2.5 and 3.1 cm submitted to a rectilinear velocity of 0.028 m/sec in a solution of 0.5% sodium bisulfite. Mass transfer coefficients in each case were indicated in the same figure.

The uptake of sulfur dioxide by cubes and parallelepipeds (French fry potato strips) was calculated using Eq. (11). Comparison of theoretical and experimental results for cubes (1 × 1 × 1 cm), with rectilinear velocities of 0.020 and 0.028 m/sec is observed in Fig. 5. Good agreement with experimental data was obtained calculating the mass transfer coefficients with Eq. (16).

In the case of French fry potato strips, uptake values were measured in parallelepipeds (1 × 1 × 7 cm) with rectilinear motion of 0.010 and 0.020 m/sec (Fig. 6). Corresponding mass transfer coefficients were indicated in the same figure. Percent

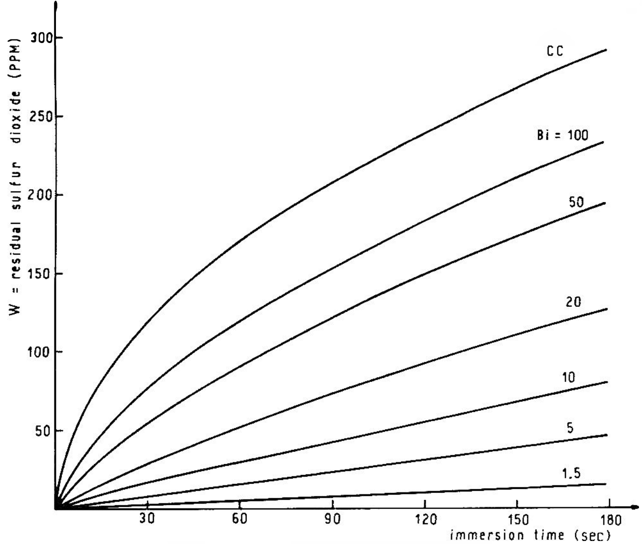

Fig. 3—Effect of Biot number on sulfur dioxide uptake in spherical pre-peeled potatoes of R = 1.1 cm. Concentration of dipping solution 0.5% sodium bisulfite. Curve CC corresponds to constant interfacial concentration. $D_e = 1.078 \times 10^{-9}$ m²/sec.

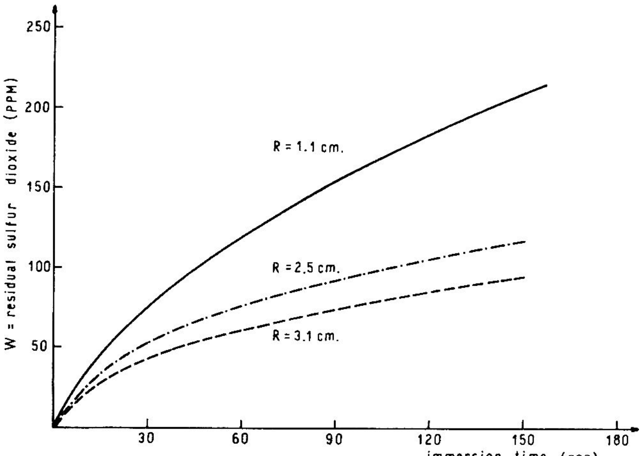

Fig. 4—Size effect on uptake values. Spherical geometry: v = 0.028 m/sec; $D_e = 1.078 \times 10^{-9}$ m²/sec; concentration of dipping solution = 0.5% sodium bisulfite — R = 1.1 cm, $k_L = 9.75 \times 10^{-6}$ m/sec; ---- R = 2.5 cm, $k_L = 6.4 \times 10^{-6}$ m/sec; --- R = 3.1 cm, $k_L = 5.77 \times 10^{-6}$ m/sec.

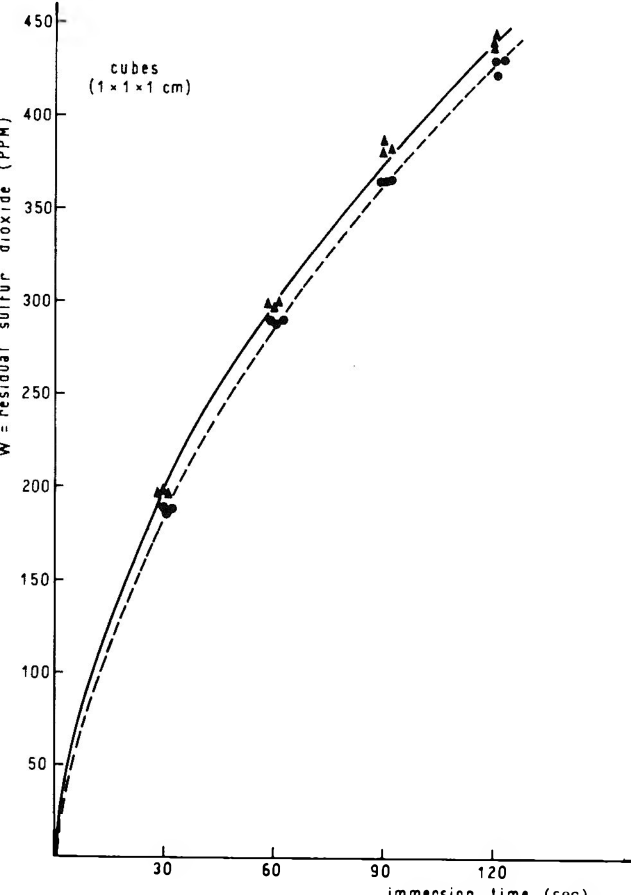

Fig. 5—Comparison of predicted and experimental data for cubes (1 × 1 × 1 cm). Concentration of dipping solution = 0.5% sodium bisulfite. Experimental ▲ v = 0.028 m/sec; ● v = 0.02 m/sec. Predicted (D_e = 1.078 × 10^{-9} m²/sec) — k_L = 1.57 × 10^{-5} m/sec; --- $k_L = 1.32 \times 10^{-5}$ *m/sec.*

deviation of the model for plane sheet solution was calculated including data for cubes and parallelepipeds (E = 1.14%).

Parallelepipeds of different length are used as potato strips in the pre-peeled potato industry. The effect of length on the amount of sulfur dioxide retained by the product was analyzed comparing results for potato samples of 1 × 1 × 5 cm and 1 × 1 × 7 cm with v = 0.020m/sec. Differences were of 0.02% at immersion times of 120 sec showing the small effect of this variable on uptake values.

Results show that the diffusive mass transfer model for porous media described adequately sulfur dioxide uptake in pre-peeled potatoes immersed in bisulfite solutions. The equations obtained can be applied to the engineering optimization of the antioxidant treatment, relating necessary levels of sulfur dioxide in the product with operating industrial conditions.

NOMENCLATURE

a, b, c	half thickness values for potato strips in x, y, z directions respectively (m)
Bi	Biot number
c	solute concentration in the solid (weight of sulfur dioxide per unit volume of potato)
c′	solute concentration in the solid (weight of sulfur dioxide per unit volume of solution; c′ = c/ε)
c_f	solute concentration in the dipping solution (weight of sulfur dioxide per unit volume of potato)
c'_f	solute concentration in the dipping solution (weight of sulfur dioxide per unit volume of solution; $c'_f = c_f/\epsilon$)
D_{AB}	molecular diffusivity (m²/sec)
D_e	effective diffusion coefficient of sulfur dioxide in the potato tissue (m²/sec)
%E	percent deviation
F	fractional uptake ($F = M_t/M_\infty$)
k_L	mass transfer coefficient at the potato fluid interface (m/sec)
L	characteristic length in plane sheet geometry (m)
M_t	amount of diffusing substance in the sample at time t
M_∞	amount of diffusing substance in the sample at infinite time
N	number of experimental data
r	distance measured from the center of the solid (m)
R	sphere radius (m)
$Re_{s,p}$	Reynolds number $Re_s = \rho v 2R/\mu$; $Re_p = \rho v L/\mu$

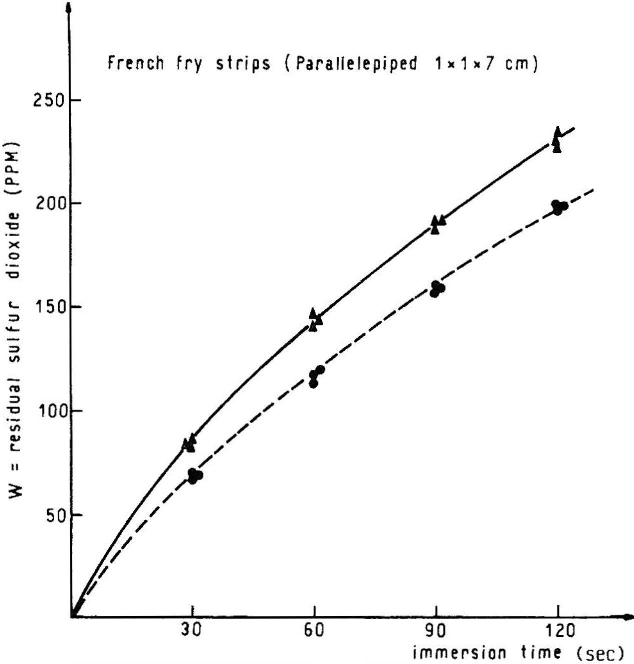

Fig. 6—Comparison of predicted and experimental data for French fry potato strips (1 × 1 × 7 cm). Concentration of dipping solution = 0.5% sodium bisulfite. Experimental ▲ v = 0.02 m/sec; ● v = 0.03 m/sec. Predicted — k_L = 0.50 × 10^{-5}m/sec; --- k_L = 0.35 × 10^{-5} m/sec.

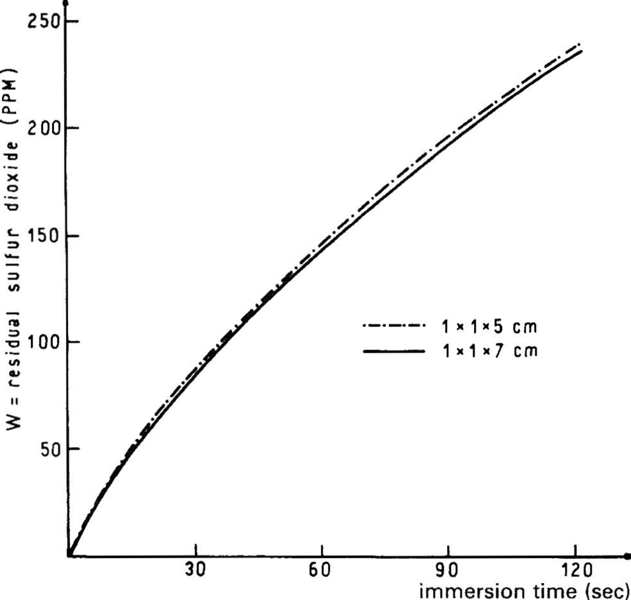

Fig. 7—Effect of French fry strips length on uptake values. v = 0.02 m/sec; ---- 1 × 1 × 5 cm; — 1 × 1 × 7 cm. Dipping solution concentration = 0.5% sodium bisulfite.

Sc	Schmidt number $Sc = \mu / \rho D_{AB}$
t	time (sec)
v	rectilinear velocity of potato cuts (m/sec)
V	potato sample volume (m^3)
W	residual sulfur dioxide (ppm)
$\alpha_{\ell,m,n}$	coefficient defined in Eq. (13)
$\beta_{m,n,\ell}$	positive roots of Eq. (12)
γ_n	positive roots of Eq. (8)
ϵ	porosity, water content on wet basis
μ	solution viscosity (kg/m sec)
ρ	density (kg/m^3)
ϕ	shape factor in Eq. (2)
Ω	tortuosity factor

Superscripts

*	dimensionless variables

Subscripts

e	experimental values
t	theoretical values

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Characterization and Distribution of Lactobacilli during Lactic Fermentation of Okra (*Hibiscus esculentus*)

P. KOTZEKIDOU and T. ROUKAS

ABSTRACT

The distribution and identification of lactobacilli during fermentation of okra were studied. Fermentation characteristics of lactobacilli isolated on MRS agar at 30°C under anaerobic conditions were presented using the API 50 CHL system. The phenogram constructed by an unweighted, pair group, arithmetic average, linkage method and by use of the Jacquard similarity coefficient (1-Sj) was used to identify nine phena. The studies showed five homolactic species initially (*Lactobacillus plantarum, Lactobacillus casei* subsp. *pseudoplantarum, Lactobacillus acidophilus, Lactobacillus leichmannii, Lactobacillus salivarius* subsp. *salicinius*) and two heterolactic species (*Lactobacillus cellobiosus* and *Lactobacillus brevis*). After 24 hr fermentation, the majority of the isolated strains were *L. cellobiosus*. During the final stage of fermentation *L. plantarum* strains dominated.

INTRODUCTION

Okra (*Hibiscus esculentus*), a vegetable cultivated in the Mediterranean region, Africa, and the Southern States of the U.S.A., is marketed in fresh, canned and frozen form. Okra contains a mucilage which is an acidic polysaccharide composed of galacturonic acid, galactose, rhamnose, and glucose (Woolfe et al., 1977). The normal pH range of okra fruit is 5.8–6.4. Therefore, a thermal process which causes maceration and change of the natural color of okra is required during canning. To overcome the problems associated with the canning of okra, fermentation of okra with starter cultures is necessary (Roukas and Kotzekidou, 1986).

Lactobacilli as starter cultures in "controlled" fermentations of vegetables have been described (Fleming and McFeeters, 1981). Even in natural fermentations, lactobacilli become established quickly. *Lactobacillus brevis* and *Lactobacillus plantarum* are responsible for the natural fermentation of many vegetables, although *L. plantarum* is by far the predominant species in most fermentations (Etchells et al., 1975).

Mundt and Hammer (1968) studied the distribution of lactobacilli on okra plants. No attempt to study the microflora during fermentation of okra fruits is reported.

The objectives of this study were to determine the distribution and identity of lactobacilli during natural fermentation of okra which is essential for the selection of starter cultures for the improvement of the quality of canned okra.

MATERIALS & METHODS

Fermentation of okra

Fresh okra (cultivar Pylea No 1) was obtained from the local produce market. Five hundred grams of okra, washed in tap water, and an equal weight of brine were added to a 5L polypropylene container. The brine contained 5% (w/v) sodium chloride and 0.005% (v/v) lactic acid. The pH of brine was adjusted to 4.6 with 0.1N NaOH. In the container, reduced oxygen tension was achieved by weighting down the okra with a steril conical flask full of water. Fermentation was carried out at 30°C. Duplicate jars were fermented and analyzed.

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Isolation of the *Lactobacillus* strains

Before fermentation and at 12, 24, 36, and 48 hr of fermentation 20g okra samples were taken under aseptic conditions. Samples of okra were homogenized and diluted in sterile 0.1% peptone water. Lactobacilli were detected on MRS agar (de Man et al. 1960; E. Merck, Darmstadt, W. Germany) after 2-3 days of incubation at 30°C under anaerobic conditions (BBL Gas Pak, H_2 + CO_2 anaerobic system, Becton Dickinson and Co., Cockeysville, USA). Plates with 30–300 colonies were selected and the colony number was determined. A number of colonies equal to the square root of the total was isolated at random according to the statistical recommendation of *Bacteriological Analytical Manual for Foods* (FDA, 1972). These colonies were transferred and purified on MRS agar. The pure cultures were incubated on MRS broth, Gram stained after 18 hr and tested for catalase reaction. The rod-shaped cells, that were catalase negative and Gram positive, were streaked on MRS agar slants and stored at 5°C for identification.

Reference strains

Lactobacillus plantarum A 164/4 and *Lactobacillus brevis* X20 were obtained from the National Institute for Research in Dairying (Reading, England).

Biochemical tests

For all *Lactobacillus* strains forty-nine tests were performed using the API 50 CHL *Lactobacillus* identification system (API System S.A., La Balme les Grottes, Montalieu Vercieu, France): fermentation of glycerol, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, β-methyl xyloside, galactose, glucose, fructose, mannose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, α-methyl mannoside, α-methyl glucoside, N-acetyl glucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose,D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconic acid, 2-keto-gluconate and 5-keto-gluconate. The reactions were observed at intervals of 3, 6, 24, and 48 hr, as recommended by the manufacturer.

Computer analysis

All tests were coded as two-state binary characters and were scored 1 (positive reaction) or 0 (negative reaction) for the purpose of coding into the computer. The taxonomic study was performed using the complement to one of the Jacquard similarity coefficient (1-Sj) and the unweighted pair group arithmetic average linkage method (Sneath and Sokal, 1973). A phenogram was constructed by the unweighted pair group arithmetic average linkage method. The computer analysis was carried out by the API Research Laboratory (La Balme les Grottes, Montalieu Vercieu, France).

Identification of the strains

The current identification program of the API Research Laboratory takes into account strains from dairy origin. For this reason, the identification of the phena was obtained using both the results of the computer analysis and a comparison of the results with the *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974).

RESULTS & DISCUSSION

THE MAJORITY of the 108 isolated *Lactobacillus* strains during fermentation of okra and the two reference strains were clustered into nine phena by the unweighted pair group arithmetic average linkage method with the complement to one of

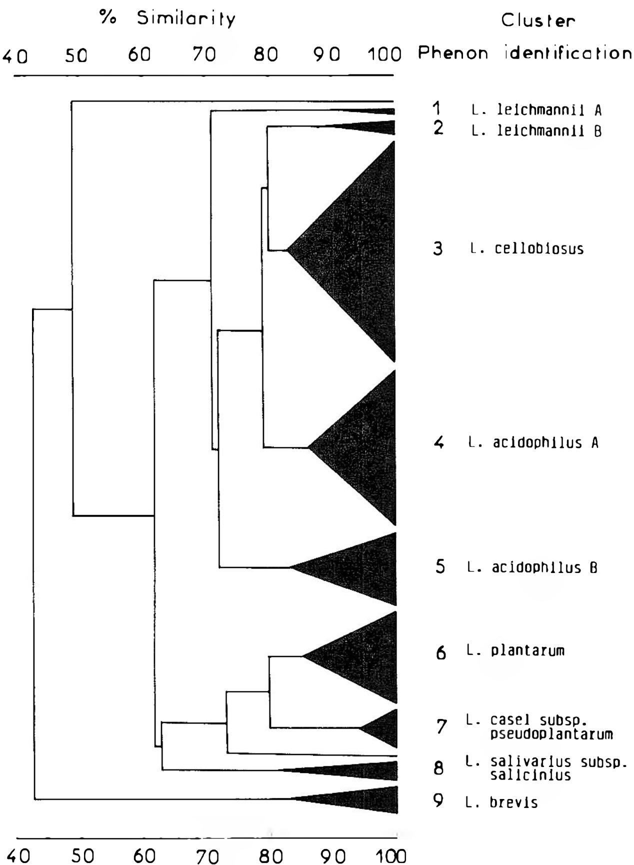

Fig. 1—Phenogram based on the unweighted pair group arithmetic average linkage method.

the Jacquard similarity coefficient (1-Sj). The results are depicted graphically in a phenogram (Fig. 1). Identification of seven phena was made, and the feature frequencies of all unidentical characteristics tested for each strain in the phena are listed in Table 1. Reactions identical for all strains were not presented in the table. The following reactions were positive for all strains: glucose, fructose, mannose, N-acetylglucosamine and maltose. The following reactions were negative for all strains: glycerol, erytritol, D-arabinose, L-xylose, adonitol, β-methyl xyloside, sorbose, rhamnose, dulcitol, inositol, α-methyl mannoside, inulin, melezitose, starch, glucogen, xylitol, D-fucose, L-fucose, 2-keto-gluconate and 5-keto-gluconate. None of the isolated *Lactobacillus* strains fermented lactose.

Phenon 1 and phenon 2 formed at 91% and 89% similarity levels, respectively, and were identified as *Lactobacillus leichmannii*. Phenon 1 contained two strains and Phenon 2 contained three strains. All strains possessed properties described for *L. leichmannii* (Buchanan and Gibbons, 1974).

Phenon 3 consisted of thirty-five strains and was identified as *Lactobacillus cellobiosus*. It formed at 83% similarity level.

Phenon 4 and Phenon 5 were identified as *Lactobacillus acidophilus* and formed at 86% and 83% similarity level, respectively. Phenon 4 contained twenty-five strains and Phenon 5 twelve strains.

Phenon 6 formed at 85% similarity level and was identified as *Lactobacillus plantarum*. Fourteen *Lactobacillus* strains were clustered with the reference strain *L. plantarum* A 164/4. Only the reference strain utilized lactose.

Phenon 7, identified as *Lactobacillus casei* subsp. *pseudoplantarum*, formed at the 94% similarity level. Seven strains were clustered in this phenon and all fermented D-tagatose.

Phenon 8 formed at 82% similarity level and was identified as *Lactobcillus salivarius* subsp. *salicinius*. It contained four strains. All strains utilized L-arabitol.

Phenon 9 was identified as *Lactobacillus brevis* and formed at 83% similarity level. Four strains isolated during fermentation of okra were clustered with the reference strain *L. brevis* X20. All strains fermented α-methyl glucoside. Two strains did not enter any of the 9 phena and remained unclustered.

From unfermented okra fruits were isolated the following lactobacilli: *L. leichmannii, L. cellobiosus, L. acidophilus and L. salivarious* subsp. *salicinius* (Fig. 2). Mundt and Hammer (1968) reported that they isolated the following lactobacilli from okra plants: *L. plantarum, L. fermenti* and *L. casei*. The species of the genus *Lactobacillus* that were isolated from okra fruits were not identical to the identified strains of okra plants. This was probably due to the geographic origin of the strains.

Shaw and Harding (1984) in a numerical taxonomic study on the microflora of vacuum-packed meats identified two groups of streptobacteria distinguishable from most of the species of streptobacteria. The pattern of fermentation of carbohydrates

Table 1—Properties of phena

	% of following strains giving positive result:								
Trait	Phenon 1 (2)[a]	Phenon 2 (3)	Phenon 3 (35)	Phenon 4 (25)	Phenon 5 (12)	Phenon 6 (15)	Phenon 7 (7)	Phenon 8 (4)	Phenon 9 (5)
L-Arabinose	0	100	89	20	0	93	0	100	80
Ribose	0	0	94	0	8	100	100	0	100
D-Xylose	100	100	100	68	100	0	0	0	40
Galactose	0	33	91	88	83	100	100	75	20
Mannitol	0	0	0	0	0	93	100	75	20
Sorbitol	0	0	0	0	0	93	86	100	0
α-Methyl glucoside	0	0	0	0	0	0	0	0	100
Amygdalin	100	67	94	100	91	100	100	25	0
Arbutin	0	100	100	100	100	100	100	100	20
Esculin	100	100	100	100	100	100	100	100	20
Salicin	50	100	20	88	91	100	100	100	0
Cellobiose	100	100	100	100	100	100	100	25	20
Lactose	0	0	0	0	0	7	0	0	0
Melibiose	0	0	91	88	100	93	0	75	100
Sucrose	100	67	100	100	100	80	100	100	80
Trehalose	100	100	94	96	91	100	100	100	20
Raffinose	0	0	91	92	83	80	0	75	20
Gentiobiose	50	0	71	0	0	100	100	50	0
D-Turanose	0	0	0	0	0	100	86	0	0
D-Lyxose	0	0	0	0	0	0	86	0	0
D-Tagatose	0	0	0	0	0	0	100	0	0
D-Arabitol	0	0	0	0	0	7	0	0	0
L-Arabitol	0	0	0	0	0	0	0	100	0
Gluconic acid	0	0	89	0	0	93	86	25	80

[a] No. of strains in phenon

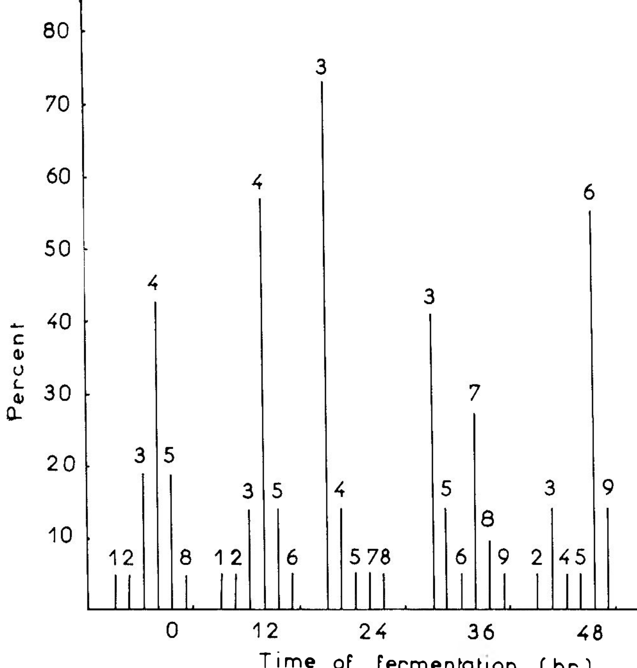

Fig. 2—Percent distribution of isolated species of the genus Lactobacillus: L. leichmannii A (1), *L. leichmannii* B (2), *L. cellobiosus* (3), *L. acidophilus* A (4), *L. acidophilus* B (5), *L. plantarum* (6), *L. casei* subsp. *pseudoplantarum* (7), *L. salivarius* subsp. *salicinius* (8), and *L. brevis* (9).

differentiated the identified groups of streptobacteria from the phena of lactobacilli isolated during fermentation of okra.

The majority of lactobacilli isolated during fermentation of okra (64% of the isolated strains) were homofermentative. One potential problem in fermentations with homofermentative organisms is the strong lactic acid flavor developed by these fermentations which may be undesirable for many people (Chen et al., 1983).

After 24 hr and 36 hr of fermentation the strains of *L. cellobiosus* were dominant (Fig. 2). Many natural food fermentations are initiated by heterofermentative lactic acid bacteria (Pederson, 1971). These bacteria produce acetic acid, ethanol, mannitol and CO_2 in addition to lactic acid. Less acid will be produced from a given amount of sugar than in homolactic acid fermentations. The amount of produced acid is significant, because with the fermentation of okra a pH value slightly below 4.5 is desired in order to preserve the natural color and to remove as much mucilage as possible (Roukas and Kotzekidou, 1986). After 48 hr of fermentation the strains of *L. plantarum* predominated (Fig. 2), while the pH of okra decreased to 4.1 and of the brine to 3.4 (Roukas and Kotzekidou, 1986). *L. plantarum* is the terminal organism in many natural lactic acid fermentations due to its high acid tolerance (Fleming and McFeeters, 1981).

This study of lactobacilli microflora during fermentation of okra was important in overcoming the problems associated with the canning of okra and in selecting the appropriate cultures for okra fermentation.

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Effects of Processing and Storage on the Folate Content of Spinach and Broccoli

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ABSTRACT

Free and total folate activities (FFA and TFA) in spinach and broccoli were determined at various processing stages. In most cases FFA was significantly lower than TFA ($P < 0.05$). Steam-blanching resulted in greater TFA retention than water-blanching. Spinach lost 50% of the TFA during canning and another 10% after storage for 3 months. Storage at −32.2°C of water blanched spinach resulted in 27% TFA loss after 3 months and 17% TFA loss for water-blanched broccoli after 8 months. For spinach and broccoli, 40% and 68%, respectively, of the TFA was in the blanch water but only 1% and 4% TFA, respectively, were in the steam-blanch condensate. When canned, 14% of the TFA in spinach immediately after processing was in the liquid in the can. This amount increased to 18% after 3 months storage.

INTRODUCTION

VEGETABLES, because of the quantities consumed, are major contributors of folate in the U.S. diet. Since many of the vegetables consumed today are available either canned or frozen, they are subjected to various processes before consumption. Processing can lead to reduced folate in a food, either by degradation, or by leaching into the processing effluents (Leichter, 1980). Folate, being water soluble, is lost during operations such as blanching and canning. Schroeder (1971) reported folate losses after canning from between 35% for spinach to 84% for mushrooms. Chen et al. (1983) observed a 14% loss of folate in microwave-blanched spinach, and a 33% loss in water-blanched spinach. In the same study, a loss of 27% folate was reported after 10 weeks at −22°C. In contrast, Malin (1977) studying effects of blanching, freezing and storage on Brussels sprouts, found that the folate was stable during all processing stages. This finding reflects minimal leaching due to the small exposed surface area of Brussels sprouts as compared to spinach. Leichter (1980) found that liquid portions of canned vegetables had folate activities comparable to those of the corresponding solid portions, due to leaching out of the vitamin into the canning medium during storage. Gami and Chen (1985) found that destruction of folate in Swiss chard followed first-order kinetics, and was most stable when stored in plastic bags and least stable in open air at 40°C.

Various studies have shown that cooking can lower the folate content of foods. Retention of folate in different meats after roasting, braising or stewing ranged from 8% to 46% (Schweigert et al., 1946). Leichter et al. (1978) found 22% retention of folate in cooked spinach, with 65% of the original folate leaching out into the cooking water. Klein et al. (1979) reported 51% retention of folate in cooked broccoli, and a later study found 77% retention in cooked spinach (Klein et al., 1981).

Although considerable data on folate content of spinach are available in the literature, most studies concerned with processing effects on folate levels have not provided comprehensive data through an entire processing scheme. Therefore, this study was designed to delineate folate changes in both spinach and broccoli through several processing steps. Specific objectives included the following: (1) to compare two common blanching treatments (water and steam) on the retention of folate in broccoli and spinach; (2) to define the role of leaching in the loss folate by following transfer of the vitamin into the blanching and canning media; (3) to determine the effects of canning (spinach only), freezing and storage on folate levels in the vegetables.

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MATERIALS & METHODS

Sources of spinach and broccoli

Fresh California spinach purchased from a local wholesale produce distributor was stored at a mean temperature of 1.1°C until prepared for processing. Samples were usually processed within 2 days after arrival at the laboratory. The time from harvest to the arrival of spinach at Athens was estimated at two to four days. During transportation, the rosettes of spinach were held together in bunches by paper covered wire and shipped in waxed fiberboard boxes.

The broccoli was also California grown and purchased from a local distributor. Broccoli was prepared as soon as it was received. The broccoli was received packed in ice in waxed fiberboard boxes.

Sample preparation

The entire spinach rosette is usually harvested by cutting the tap root at the soil surface and inevitably carries some mud and sand. In general, it is not washed before long-distance shipping since doing so might accelerate its decay. Preparation of the spinach was accomplished by washing with tap water first to get rid of most of the mud and sand and then washing with deionized water. The washed spinach was spread out in large and shallow draining baskets with intermittent stirring to dry uniformly. When the spinach was air-dried back to the point of no visible moisture, it was considered as a raw sample and ready for subsequent processing.

Fresh broccoli is usually harvested by cutting with 8–10 in. (20–25 cm) of stem. It is very perishable and usually cooled right after harvest at temperatures of 0.6–2.8°C. Preparation of the broccoli included removal of the leaves and dividing individual flower heads. The small heads of broccoli were washed with tap water and then with deionized water. Like spinach, the washed broccoli was drained and air-dried.

Processing

Blanching. Commercial blanching of spinach is accomplished either by immersing raw product in water for 3 min at 100°C or for 6 min at either 85°C or 100°C (Lopez, 1981). To approximate industrial conditions, an intermediate process was chosen for this study (4 min at 100°C). To water-blanch the vegetables, spinach or broccoli was immersed in boiling water in a steam kettle in a 1:7 ratio, using 4 kg in 28L water for spinach, and 1 kg in 7L of water for broccoli. This ratio closely approximates that recommended in USDA Bulletin #10 (Anonymous, 1974) for the blanching of vegetables. After boiling commenced, the vegetables were blanched for 4 min. The blanched vegetables were cooled immediately by immersion in ice water for 1 min and allowed to drain. The blanching effluents were collected and reconstituted to their original volumes.

Steam-blanching was carried out by placing a 200g vegetable sample in a plastic colander on a perforated platform in a home style canner containing 500 mL boiling water. After a 4-min blanch, the vegetables were cooled by immersion in cold water and drained. The condensate was collected and reconstituted to 500 mL.

Canning. Following the water-blanched treatment, 350g drained spinach were placed in 500 mL jars with 60 mL deionized water, sealed and processed in a retort at 122°C for 54 min at 15–18 psi (Lopez,

Table 1—Free and total folate in spinach at different stages of processing

Product	Free (μg/100 g) wet weight basis	Total (μg/100 g) wet weight basis	Retention[e] (%)	% TFA in Processing Media
Spinach				
Raw	$160.9_A \pm 43.7^a$	$251.4_A \pm 52.9^b$		
Water blanched	$39.1_B \pm 35.8^a$	$42.5_B \pm 18.2^a$	17	
Steam blanched	$34.9_B \pm 14.7^a$	$146.7_C \pm 40.5^b$	58	
Canned	$7.1_C \pm 4.2^a$	$21.2_D \pm 3.9^b$	50 (12)	
Canned/stored (3 months)	$1.4_D \pm 0.4^a$	$17.0_D \pm 1.6^b$	40 (10)	
Frozen (3 months)	$6.0_C \pm 0.6^a$	$30.8_B \pm 3.3^b$	72 (12)	
Processing Media				
Water blanch effluent	$2.0_E \pm 1.8^c$	$14.5_E \pm 8.1^d$		40
Steam blanch condensate	$0.8_E \pm 0.5^c$	$1.1_F \pm 0.5^c$		1
Canning medium	$7.0_F \pm 1.0^d$	$34.0_G \pm 5.0^d$		14
Canning medium (3 months)	$12.3_F \pm 0.8^c$	$44.6_G \pm 1.7^d$		18

[a-d] Values represent Means ± S.D. Means across each row with the same superscripts are not significantly different (P < 0.05).

[A-G] Means down each column with the same subscript are not significantly different (P < 0.01).

[e] Retention based on total folate in the product at each processing step. Numbers in parentheses indicate retention values relative to raw sample.

1981). Jars were cooled by means of water sprays and placed in storage in the dark at 21°C. Samples of spinach as well as the canning medium were an analyzed immediately after canning and again after 3 months storage.

Freezing. After water-blanching, 100g samples of spinach and broccoli were packed in heat-sealed unevacuated freezer pouches, which were stored at −32.2°C in a blast freezer for 3 months for spinach and 8 months for broccoli.

Analysis. Samples of spinach, broccoli, and processing media were analyzed at every processing step for free and total folate using a *Lactobcillus casei* microbiological procedure adapted from Yamada (1979) and Khalsa (1982). This treatment incorporates 4-hr incubation with a proteolytic enzyme, Pronase® (Calbiochem-Behring Corp., LaJolla, CA), in addition to the regular conjugase treatment. This method would enable any protein-bound folate present to be liberated and become available for the growth of the microorganism. Drained samples were weighed into glass beakers and homogenized in ascorbic acid-phosphate buffer (0.1M sodium phosphate Na_2HPO_4, 1% ascorbic acid, pH 6.8). Acetone washed chicken pancreas (Difco Laboratories, Detroit, MI) (4 mg/g sample) was added. The mixture was incubated overnight at 37°C. The next day Pronase® (6 mg/g sample) was added and incubated 4 hr at 37°C. This was followed by steaming for 5 min at 100°C and centrifuging (7500g, 5min). The clear extract was used for microbiological analysis. Enzyme controls were run concurrently with the assay. Recovery of added pteroylglutamic acid in spinach was 95.6 ± 0.8% and in broccoli 91.5 ± 1%.

Four replicate values were obtained from each of two independent trials. One-way analysis of variance was used to test for significant differences between treatments (Snedecor and Cochran, 1980).

Retention values and total folate activity (TFA) values were calculated based on the total amount of folate contained in the product at the beginning of each processing step. Murphy et al. (1975) described two ways of calculating retention. Retention calculated on a dry weight basis is called apparent retention, which is defined as the amount retained based on nutrient content per unit dry matter before and after processing. True retention is defined as the amount retained based on total nutrient contents of known weights of food before and after processing. The retention values calculated in this study represent true retention as defined by Murphy et al. (1975). The following formulas were used:

$$\%\ \text{Retention} = \frac{\mu g/g\ \text{TFA in processed sample} \times g\ \text{sample after processing}}{\mu g/g\ \text{TFA in sample before processing} \times g\ \text{sample before processing}} \times 100$$

$$\%\ \text{TFA in processing media} = \frac{\mu g\ \text{TFA in total liquid medium after processing}}{\mu g\ \text{TFA in total vegetable sample before processing}} \times 100$$

RESULTS & DISCUSSION

Spinach

Data on folate content of spinach are presented in Table 1. Raw spinach contained 251.4 μg/100g total folate activity (TFA). The value reported in USDA Handbook 8-11 (Haytowtiz and Matthews, 1984) is 174.4 μg/100g. Water-blanched spinach retained 17% TFA, with 40% TFA present in the blanch effluent. Leichter et al. (1978) found a 21.8% retention between raw and cooked spinach, with 65% leaching out into the cooking water. Chen et al. (1983) reported a 67% TFA retention in water-blanched spinach. The blanching procedure used in their study differed from ours in that the spinach-to-water ratio was higher (1:1.6) than that used in this study (1:7), and the blanching time was shorter (3 min compared to 4 min). A longer blanch with more water present would be expected to lead to lower retention values. Klein et al. (1981) found a 77% retention of folate in spinach cooked conventionally. The spinach-to-water ratio was very high, 4:1, thus, less folate would be expected to leach out into the water.

Steam-blanched spinach retained 58% TFA, with 1% leaching out into the condensate. The higher retention reflects the absence of leaching by water that takes place in water-blanching. No comparable data on steam-blanching of spinach was found in the literature. In both water-blanched and steam-blanched spinach, approximately 60% TFA could be accounted for in the combined sample and blanching effluent or condensate. Apparently, the spinach blanch medium was more conducive to folate destruction than the broccoli blanching medium since no large net loss of folate was observed either in water-blanched or steam-blanched broccoli, which has a comparatively much smaller surface area. Canned spinach retained 50% from the water-blanched sample, and 40% after 3 months storage. The canning medium contained 14% of the TFA, rising to 18% after 3 months. The loss during the canning procedure was significant (P < 0.01), while loss during canned storage was not.

The TFA value for canned spinach obtained in this study was considerably lower than the 129 μg/100 g TFA in canned spinach reported by Leichter (1980) who assumed that no folate destruction occurred during the canning process. However, the material that was filled into the cans that were analyzed was not assayed for folate activity. Values for comparison were instead obtained from the literature. The method of blanching used before canning was also not specified. The higher folate activities reported by Leichter (1980) could be due to a steam or microwave-blanch being applied prior to canning rather than a water-blanch used in this study. The more severe water-blanch treatment would lead to lower retention values, especially when calculated as a percentage of raw sample content. Leichter (1980) found folate activities in the liquid portions of canned spinach comparable to those in the corresponding solid portions.

Frozen stored spinach retained 72% of the TFA present in the water-blanched spinach after 3 months at −32.2°C. The 28% loss agrees well with the 27% loss reported by Chen et al. (1983) after storing spinach at −22°C for 10 wk. The loss on freezing was not significant (P < 0.01). Free folate activity (FFA) in raw spinach was 160.9 μg/100g, droping to 39.1 μg/100g and 34.9 μg/100g in water-blanched and steam-blanched

Table 2—Free and total folate in broccoli at different stages of processing

Product	Free (μg/100 g) Wet Weight Basis	Total (μg/100 g) Wet Weight Basis	Retention[e] (%)	% TFA in Processing Media
Broccoli				
Raw	$57.5_A \pm 18.7^a$	$102.2_A \pm 31.0^b$		
Water blanched	$2.3_B \pm 3.4^a$	$30.3_B \pm 5.8^b$	30	
Steam blanched	$4.1_B \pm 6.3^a$	$92.5_C \pm 20.3^b$	91	
Frozen (8 months)	$1.3_B \pm 0.4^a$	$25.0_B \pm 1.8^b$	83 (25)	
Processing Media				
Water blanch effluent	$0.4_D \pm 0.5^c$	$10.0_D \pm 2.6^d$		68
Steam blanch condensate	$0.3_D \pm 0.3^c$	$1.6_E \pm 1.3^d$		4

[a-d] Values represent Means ± S.D. Means across each row with the same superscript are not significantly different ($P < 0.05$).
[A-E] Means down each column with the same subscript are not significantly different ($P < 0.01$).
[e] Retention based on total folate in the product at each processing step. Numbers in parentheses indicate retention values relative to raw sample.

spinach, respectively. Raw spinach contains a conjugase enzyme which can deconjugate pteroylglutamates, leading to higher FFA values if the enzyme is not inactivated prior to initiation of extraction of the vitamin (Leichter et al., 1979; Chen et al., 1983). The high FFA value in raw spinach indicated the presence of endogenous conjugases, and the low FFA values after both water and steam blanching may indicate that endogenous conjugases were effectively destroyed after either blanching procedure, although the decrease in free folate could also be due to heat destruction and leaching loss.

Broccoli

Data on folate content of broccoli are presented in Table 2. Raw broccoli contained 102.2 μg/100g TFA. The value reported in USDA Handbook 8-11 (Haytowitz and Matthews, 1984) is 78 μg/100g. TFA retention in water-blanched broccoli was 30%, with 68% of the TFA in the blanch effluent. This compares favorably with results obtained by Leichter et al. (1978) who reported a 38% retention of folate in cooked broccoli with 69% in the cooking water. Steam-blanched broccoli retained 91% of the TFA, with 4% in the condensate. After 8 months frozen storage at −32.2°C, broccoli retained 83% of the TFA present in the water-blanched product. The loss upon freezing was not significant ($P < 0.01$). As in the case of spinach, FFA declined drastically from 57.5 μg/100g in the raw sample to 2.3 μg/100g and 4.1 μg/100g in water and steam blanched samples, respectively. Thus, both water and steam blanching effectively destroy endogenous conjugases that increase measurable FFA during the folate extraction procedure.

In conclusion, this study provided quantitative information on folate loss in spinach and broccoli through several stages of processing. Spinach which has a larger surface area than broccoli had higher losses at every processing step. Steam-blanching led to much higher TFA retention than water-blanching. A significant ($P < 0.01$) loss occurred during the canning process while no significant loss occurred during canned or frozen storage. The data clearly show the role of leaching in the loss of a water-soluble vitamin during blanching and into the canning medium. Draining of the canning medium before consumption would result in a significant loss of folate to the consumer.

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Functional Properties of Freeze-Dried Powders of Unfermented and Fermented Aqueous Extracts of Legume Seeds

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ABSTRACT

Functional properties of freeze-dried powders made from aqueous extracts of cowpea, peanut and soybean seeds were compared with those of a commercial cultured buttermilk powder product. Powders of seed extracts fermented with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were also analyzed. The color of legume powders was similar to that of the commercial buttermilk product, and fermentation had a beneficial effect. Emulsion capacities of powders prepared from extracts were superior to the commercial product, as were foam capacity and stability. The nitrogen solubility profiles of powders from unfermented extracts were similar to those of powdered buttermilk; however, solubility of nitrogen in fermented extracts was less than that of unfermented products. The viscosity of rehydrated fermented powders was less than that of the controls but greater than that of the commercial product. The water adsorption capacity of various seed extract powders was similar, regardless of fermentation treatment.

INTRODUCTION

WHEN NEW FOOD INGREDIENTS are developed, it is important to evaluate how they will perform in foods. Ingredients that contain substantial levels of protein are often tested most vigorously, since proteins can greatly influence functionality in many food systems.

Investigations have been reported describing functional properties of legume proteins modified by various means (Puski, 1975; Beuchat et al., 1975; Quinn and Beuchat, 1975). Functional properties of several types of legume flours have been evaluated (Lawhon et al., 1972; Sosulski et al., 1976; McWatters and Cherry, 1977). No reports are available, however, comparing functional properties of freeze-dried powders made from unfermented and fermented aqueous extracts of legume seeds. As described in a companion report (Schaffner and Beuchat, 1986), aqueous extracts of cowpea, peanut and soybean can be fermented with several species of lactic acid bacteria to produce yogurt-like products. The study reported here provides information concerning the functional properties of freeze-dried powders made from these unfermented and fermented extracts.

MATERIALS & METHODS

Sample preparation

The procedure for preparing aqueous extracts (legume milks) of cowpeas, peanuts and soybeans was described in another report (Schaffner and Beuchat, 1986). The procedure followed in the present study was the same except that the quantity of legume milk and the inoculum volume were increased. A single batch consisted of 8L of legume milk and 80 mL of inoculum. Batch fermentation (unagitated) with *Lactobacillus bulgaricus* NRRL B-1909 and *Streptococcus thermophilus* was carried out at 37°C for 24 hr. Immediately following fermentation, the samples were frozen at −18°C and freeze-dried. The freeze-dried materials were then milled in a Retsch microjet mill (F. Kurt Retch GmbH and Co., Germany, model ZM1) using a 1.0 mesh screen. After milling the samples were stored in sealed containers at 5°C until tested. For purposes of comparison, freeze-dried unfermented legume extracts and a commercial freeze-dried cultured buttermilk powder (CBMP) were also subjected to functional property analysis.

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Sample testing

Color. The color of the freeze-dried powders was measured using a Gardner colorimeter (Pacific Scientific, Gardner Laboratory Division, Bethesda, MD, model XL800) with a XL845 circumferential sensor. The reference plate used was of yellow hue with color coordinate values of $L = 79.56$, $a_L = -2.17$ and $b_L = 22.98$.

Emulsion capacity. Three quantities of powder (0.5, 1.0 and 3.0g) were added to an improvised blender jar and mixed with 25 ml of distilled water. Peanut oil (Nugget Brand, Atlanta, GA) was added at the rate of 0.3 mL/sec and blended at low speed. The emulsion capacity was defined as the minimum amount of oil needed to reach the emulsion breakpoint. The emulsion breakpoint was subjectively defined as a sudden loss in the apparent viscosity of the emulsion. Emulsion data were analyzed for significant differences ($P \leq 0.05$) by Duncan's multiple range test.

Foam capacity and stability. After preliminary studies of several techniques used to generate a measurable foam, a procedure based on that reported by Yasumatsu et al. (1972) was selected as the best technique for evaluating freeze-dried powders. One gram of freeze-dried powder was added to 75 mL of distilled water in a 100-mL graduated cylinder and vigorously shaken for 30 sec. The resulting volume (foam plus liquid) was recorded initially and at times up to and including 30 min.

Gel electrophoresis. The disc polyacrylamide gel electrophoresis method reported by Chiou et al. (1985) was used to analyze changes in protein banding patterns that occurred as a result of pasteurization and fermentation of the legume milks. The standards used were carbonic anhydrase (molecular weight, 29,000), bovine albumin (66,000), phosphorylase B (250,000), and β-galactosidase (515,000).

Nitrogen solubility. One gram of freeze-dried powder was added to 50 mL distilled water. The pH of this suspension was adjusted with either 0.1N sodium hydroxide or 0.1N hydrochloric acid. Samples were adjusted to pH 2, 4, 6, 8, and 10 over a 1-hr period and then centrifuged for 10 min at $10,000 \times g$. The aqueous solution was decanted and analyzed for nitrogen content by the Kjeldahl method (AOAC, 1975).

Viscosity of rehydrated powders. Ten grams of freeze-dried powder were added to 30 mL of distilled water at 22°C and mixed thoroughly. The viscosity of a portion of this mixture was determined with a digital Brookfield viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA, model HATD) using cylindrical spindle HA-21.

Water adsorption. The equilibrium moisture contents of the freeze-dried powders were determined using constant relative humidity environments similar to the one described by Chhinnan and Beuchat (1985). Saturated salt solutions were placed in sealed glass containers and allowed to equilibrate at 21°C; equilibrium relative humidity (ERH) values were estimated from those reported by Rockland (1960). The salts and their ERH values used were LiCL (12%), $MgCl_2$ (33%), $Mg(NO_3)_2$ (52%), NaCl (75%) and K_2SO_4 (97%). Duplicate 1-g samples were weighed and placed in containers. At the end of 10 days, samples were removed and immediately weighed. From these data, weight gains and percent moisture were calculated.

RESULTS & DISCUSSION

Color

The color of unfermented and fermented freeze-dried legume milk powders was darker than that of CBMP, as can be

Table 1—Tristimulus color values of freeze-dried legume milk powders and CBMP

Substrate	Fermentation treatment	Tristimulus values		
		L	*a*	*b*
Cowpea	unfermented	70.31	0.31	13.87
	L. bulgaricus	74.47	1.61	16.54
	S. thermophilus	73.83	1.51	15.70
Peanut	unfermented	49.03	3.26	11.05
	L. bulgaricus	52.27	4.33	13.01
	S. thermophilus	50.22	4.52	12.75
Soybean	unfermented	63.55	2.17	20.50
	L. bulgaricus	64.40	3.27	21.61
	S. thermophilus	65.44	2.30	21.39
CBMP	—	94.72	−2.86	15.14

Table 2—Emulsion capacities of freeze-dried legume milk powders and CBMP

Treatment	g of powder/ 25 mL water	Product type			
		Cowpea	Peanut	Soybean	CBMP[j]
Unfermented	0.5	$^{a}133.0^{c}$	$^{a}174.9^{b}$	$^{a}179.3^{a}$	118.4^{d}
	1.0	$^{e}86.6^{b}$	$^{d}99.3^{a}$	$^{c}100.4^{a}$	69.3^{c}
	3.0	$^{h}40.3^{ab}$	$^{h}39.3^{b}$	$^{f}42.8^{a}$	29.9^{c}
Fermented with *L. bulgaricus*	0.5	$^{b}130.1^{b}$	$^{a}172.8^{a}$	$^{b}116.6^{c}$	118.4^{c}
	1.0	$^{f}74.9^{b}$	$^{e}95.0^{a}$	$^{d}72.6^{b}$	69.3^{c}
	3.0	$^{i}30.5^{b}$	$^{h}37.5^{a}$	$^{g}29.8^{b}$	29.9^{b}
Fermented with *S. thermophilus*	0.5	$^{d}103.8^{c}$	$^{b}150.4^{a}$	$^{b}117.4^{b}$	118.4^{b}
	1.0	$^{g}70.4^{b}$	$^{f}85.1^{a}$	$^{e}70.5^{b}$	69.3^{b}
	3.0	$^{i}29.1^{b}$	$^{i}34.7^{a}$	$^{g}30.3^{b}$	29.9^{b}
CBMP[j]	0.5	$^{c}118.4$	$^{c}118.4$	$^{b}118.4$	
	1.0	$^{g}69.3$	$^{g}69.3$	$^{e}69.3$	
	3.0	$^{i}29.9$	$^{i}29.9$	$^{g}29.9$	

[a-i] Values shown are mL of oil emulsified/g of powder. Numbers in columns are significantly different ($P \leq 0.05$) if not preceded by the same superscript letter; numbers in rows are significantly different ($P \leq 0.05$) if not followed by the same superscript letter.

[j] Emulsion capacities for CBMP are included for comparison purposes only. This product was not derived from legume seed or produced by fermentation procedures described in the Materials & Methods section.

seen by the relative L values listed in Table 1. Fermentation caused an increase in L values, indicating that products became lighter in color. The legume powders were also slightly redder as indicated by their positive *a* values, while the CBMP was slightly greener (with a negative *a* value); fermentation caused an increase in redness. The soybean powder was the most yellow, having the highest *b* value. The cowpea and peanut powders exhibited about the same degree of yellow color as did CBMP and fermentation did not significantly influence *b* values for any of the legume products evaluated. Overall, little difference was noted between color changes induced by *L. bulgaricus* and *S. thermophilus*.

The most important pigments in the coloration of oilseed protein products are the plant phenols (Blouin et al., 1981), and it is likely that these compounds also contribute significantly to coloration of fermented legume milks and freeze-dried powders produced from them. If some or all of these compounds could be removed by methods similar to those proposed by Blouin et al. (1981), products closer in color to that of the CBMP should be obtained.

An interesting observation is made when the L values of the liquid fermented legume milks (Schaffner and Beuchat, 1986) are compared to the L values of the freeze-dried products. Upon freeze drying, the L values of the soybean and peanut products decreased dramatically. In contrast, when the cowpea product was freeze-dried, the L values actually increased slightly. From the viewpoint of color acceptability of freeze-dried powders, the cowpea product came the nearest to CBMP even though as a liquid product it is the darkest in color.

Emulsion capacity

An examination of emulsion capacities revealed that the amount of dried powder suspended in 25 mL of water greatly affected values (Table 2). As the amount of powder in the test system was increased from 0.5 to 3.0g, the emulsion capacity was decreased, regardless of the source of powder. Differences in emulsion capacities between various seed types were very apparent when 0.5g of powder was used. These differences were less apparent when 1.0 or 3.0g were present in the test mixture. In the following discussion it should be noted that when differences are mentioned, reference is being made to the 0.5-g level unless stated otherwise.

Overall, the oil emulsion capacity of legume powders exceeded that of CBMP. This observation may result in part from differences in powder composition as well as particle size. In the present study, particle size of legume powders and CBMP was not measured. However, there may have been differences in particle size among test materials and, as shown by Ramanathan et al. (1978), particle size can have an effect on emulsion capacity.

Unfermented peanut and soybean powders as well as powders which had been prepared from respective extracts fermented with *S. thermophilus* had better emulsion capacities than cowpea powders. This may be due to the higher lipid content in peanut and soybean powders (Schaffner and Beuchat, 1986). The presence of lipids in the dried powder may facilitate the incorporation of the added oil into a stable emulsion. Differences in metabolic activities of *L. bulgaricus* and *S. thermophilus* may also have had an effect on emulsion capacities of various legume powders.

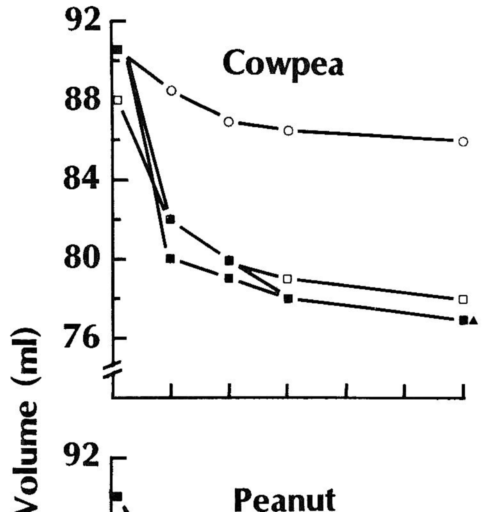
Crenwelge et al. (1974) noted that comparison of emulsion capacity of various materials is difficult and differences in results may occur because of technique rather than actual product differences. However, some useful comparisons can be made. McWatters and Cherry (1977) reported that acid conditions reduced emulsion capacities of several legume flours. This observation was confirmed in the present study. Fermentation and proteolytic enzymes have been reported to increase emulsification capacity of peanut flour (Quinn and Beuchat, 1975; Beuchat et al., 1975; Beuchat, 1977) and sunflower meal (Canella et al., 1984). The reason for the conflict in observations may be one of technique, as mentioned above, or biochemical changes induced by pretreatment and pH of the emulsion mixture. The proteolytic activities of lactic acid bacteria are characterized as weak (Law and Kolstad, 1983) in contrast to those exhibited by many fungi and to those of relatively pure proteases examined by Beuchat (1977).

Foam capacity and stability

Most of the freeze-dried powders prepared from the legume milks had both greater foam capacity and stability before fermentation when compared with CBMP. All the freeze-dried legume milk powders exhibited about the same initial increase in volume upon shaking; however, the relative stabilities of the foams were quite different (Fig. 1). The most stable foam was that of unfermented freeze-dried cowpea milk, followed by unfermented milks made from peanuts and soybeans, in that order. In every instance, fermentation reduced the foam stability of the freeze-dried powder. One explanation for these results is that acid coagulation of the proteins in the legume milks rendered them denatured and unable to retain structural integrity in foams. As noted by Yasumatsu et al. (1972), the presence of fat can have a detrimental effect on foam expansion and stability. This factor may also be influencing relative foam stabilities of the three legume products. Unfermented freeze-dried cowpea milk powder, which has a very low fat content compared to the other two legume milk powders, had the greatest foam stability.

Gel electrophoresis

Pasteurization and fermentation resulted in changes in disc gel electrophoretic patterns of the freeze-dried legume milk proteins. Pasteurization caused changes in patterns of each milk in slightly different ways. The cowpea milk proteins apparently became smaller in size upon heat treatment as shown by their



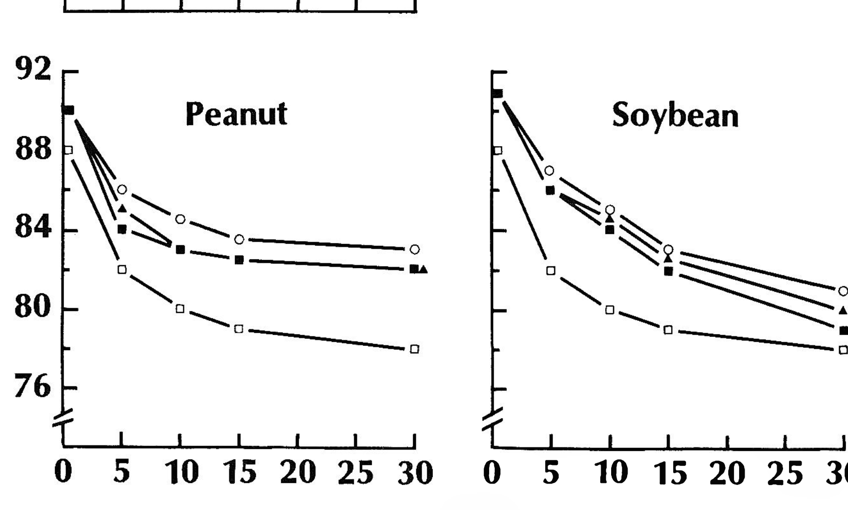


Fig. 1—Foam capacity and stability of unfermented and fermented freeze-dried legume powders and CBMP. Key: □, cultured buttermilk powder; ○, unfermented seed extract, ▲, fermented with L. bulgaricus; and ■, fermented with *S. thermophilus.*

advanced migration relative to the unpasteurized proteins. Pasteurization of peanut and soybean milks resulted in proteins with higher molecular weights as evidenced by a decreased distance of migration.

The effect of fermentation upon the banding patterns varied according to the source of milk and the bacterial strain used. In the cowpea product, there was considerable reduction in the amount of protein soluble in the electrophoresis buffer. This was most likely due to irreversible acid coagulation of proteins, which renders many of them insoluble. In the fermented products, the proteins may have originated from the seeds or from the fermentation organisms. However, most of the bands appearing in gels can be attributed to seed origin.

Coagulation and precipitation of the proteins in peanut milk fermented with *L. bulgaricus* were less extensive than in cowpea milk. Proteins had a range of molecular weights from 515,000 to less than 29.000. The most prominent band was at about 29,000 M.W. but other bands could be seen having molecular weights of 250,000, 66,000, between 66,000 and 29,000, and less than 29,000.

The electrophoretic pattern produced in peanut milk fermented with *S. thermophilus* contained no prominent bands. One reason for this may be that more protein had been irreversibly coagulated because of the greater amount of acid produced by *S. thermophilus* compared to *L. bulgaricus* in peanut milk.

The effect of fermentation on the protein electrophoretic patterns of soybean milk was similar to that of the other two milks in that the amount of protein that was soluble in the electrophoresis buffer was less than that in unfermented milk. The electrophoretic pattern of the fermented soybean milk was the least detailed of the three fermented legume milk products. Only two bands could be distinguished in a region indicating molecular weights slightly higher than 29,000.

Canella et al. (1984) showed a decrease in electrophoretic band intensity of sunflower meal when fermented. Cherry and McWatters (1981) reported that heating peanut meal results in a change in electrophoretic patterns similar to the effect observed in pasteurized peanut milks in this study. In the same article they also reported changes in electrophoretic patterns of soybean and peanut flours when the pH was lowered to 4.0. The electrophoretic patterns shown by Beuchat et al. (1976) for enzymatically hydrolyzed peanut flour do not, however, agree with results reported here. But it should be noted that the system studied by those investigators involved using proteolytic enzymes to promote protein hydrolysis. As mentioned previously, lactic acid bacteria are only weakly proteolytic and hence are not likely to cause the same effects on protein as a purified proteolytic enzyme.

Nitrogen solubility

The nitrogen solubility profiles of the unfermented seed extracts (Fig. 2) are typical and similar to many such profiles reported in the literature for legume flours and proteins in general. The nitrogen solubility profiles of the fermented extracts do not appear similar to those reported in the literature for various types of fermented plant products (Quinn and Beuchat, 1975; Shieh et al., 1982; Canella et al., 1984). While results presented here and elsewhere show that fermentation has the effect of flattening the nitrogen solubility curves over a wide pH range, indicating less change in solubility with a change in pH, the relative amounts of soluble nitrogen are very different. Data plotted in Fig. 2 illustrate that fermentation almost universally reduces the nitrogen solubility at all pH values, while data presented in other reports show the opposite effect, i.e., fermentation increased nitrogen solubility at almost all pH values. These seemingly incongruous results can be

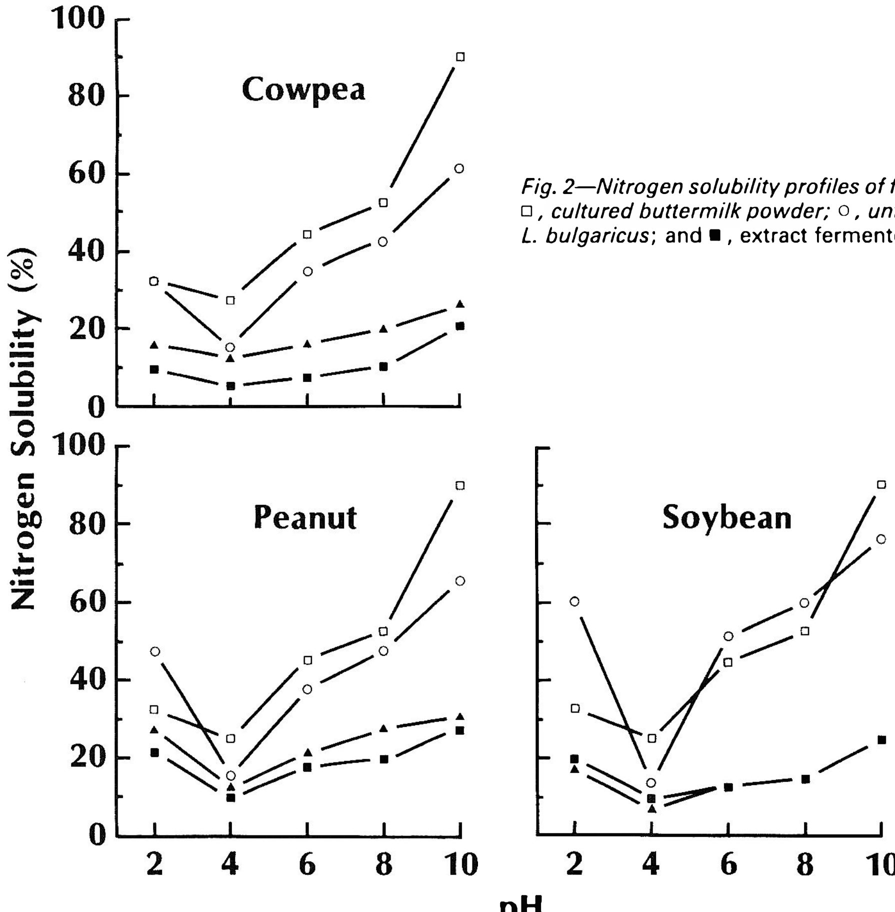


Fig. 2—Nitrogen solubility profiles of freeze-dried legume powders and CBMP. Key: □*, cultured buttermilk powder;* ○*, unfermented extract;* ▲*, extract fermented with* *L. bulgaricus*; and ■, extract fermented with *S. thermophilus*.

rationalized. The fermentation systems examined by others involved fungal fermentation or fermentation with naturally occurring inocula. In such systems proteolytic activity is likely to be extensive, resulting in more free amino acids and smaller peptides which are more soluble than the proteins from which they originated. However, lactic acid bacteria are only weakly proteolytic, and large quantities of acid are produced. Acid may irreversibly coagulate some proteins and render them less soluble over a wide pH range. The influence of acid on protein solubility is documented by McWatters and Cherry (1981) and also noted by Pour-El (1981).

The nitrogen solubility profile of CBMP closely resembled that of the unfermented seed products. Since CBMP was supposedly composed entirely of cow milk, one would not expect such a result. Upon examination of the product label, reasons for this observation were more easily explained. The product, in addition to containing dried cultured buttermilk powder, also contains sweet dairy whey and sodium caseinate. The proteins in these two components are not denatured and hence show more typical protein solubility patterns.

Viscosity of rehydrated powders

When the freeze-dried powders were rehydrated, their viscosities varied considerably (Table 3). All unfermented and fermented legume milks had viscosities much greater than that of CBMP. The probable variation in particle size and method of drying as well as composition would be expected to contribute to this difference. The cowpea powders gave the greatest viscosities when rehydrated, followed by soybean and peanut powders. The viscosities of unfermented powders were greater than those of fermented powders and, considering powders of a given seed type, viscosities of products fermented with *L. bulgaricus* were greater than those of products fermented with *S. thermophilus*.

Table 3—Viscosity of rehydrated freeze-dried legume milk powders and CBMP

Substrate type	Fermentation treatment	Viscosity (centipoise)
Cowpea	unfermented	912
	L. bulgaricus	340
	S. thermophilus	273
Peanut	unfermented	232
	L. bulgaricus	160
	S. thermophilus	127
Soybean	unfermented	277
	L. bulgaricus	191
	S. thermophilus	168
CBMP	—	25

The effect of acid pH on the viscosities of legume flour emulsions was to reduce viscosity (McWatters and Cherry, 1977). A similar phenomenon may be occurring in the present study in the case of rehydrated fermented milk powders which were at reduced pH compared to respective unfermented controls. The content of protein in the legume milk products may have also influenced the viscosities of the rehydrated powders. As reported previously (Schaffner and Beuchat, 1986), the highest concentration of protein was present in the cowpea product with less in the soybean product and still less in the peanut product. The cowpea powder had the greatest viscosity when rehydrated and the peanut powder had the least.

Water adsorption

The ability of the freeze-dried powders to adsorb water at different relative humidities is similar to that of peanut flour fermented by various fungi (Quinn and Beuchat, 1975). The freeze-dried powders made from cowpea and soybean milks exhibited responses similar to those of CBMP (Fig. 3). The

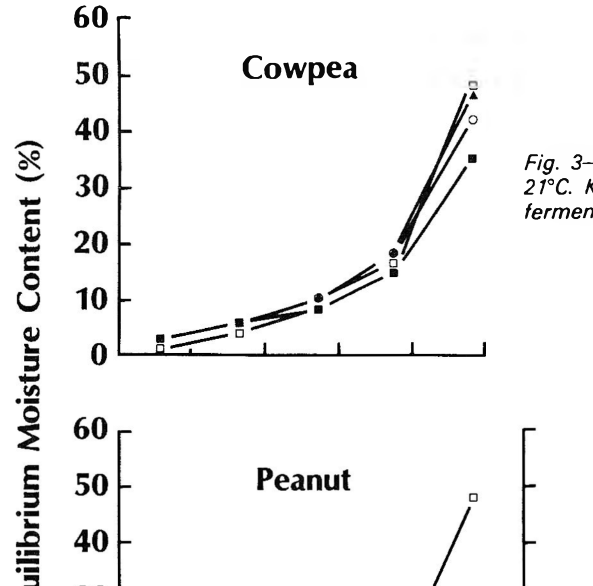


Fig. 3—Water sorption isotherms of freeze-dried legume powders and CBMP at 21°C. Key: □ , *cultured buttermilk powder;* ○ , *unfermented extract;* ▲ , *extract fermented with L. bulgaricus,* and ■ , extract fermented with *S. thermophilus.*

ability of the freeze-dried peanut powder to adsorb moisture, however, was much less than that of the other powders. One possible reason for this can be deduced from the composition of the test milks from which powders were prepared (Schaffner and Beuchat, 1986). While the cowpea milk contained 0.1% lipid and the soybean milk contained 0.60%, it is the peanut milk that contains by far the most (1.60%). This high level of lipid, which is nonpolar and hence does not have affinity for water, may reduce the ability of the freeze-dried peanut milk powder to bind water. It appears that fermentation did not have a great effect on the ability of the powders to take up water, at least under the time and temperature conditions used in this study.

SUMMARY & CONCLUSIONS

FERMENTATION of aqueous extracts of legume seeds with *L. bulgaricus* and *S. thermophilus* resulted in substantial change in functional properties. The color of unfermented and fermented freeze-dried extracts was darker than that of CBMP. The freeze-dried cowpea product was closest in color to the CBMP. Fermentation appeared to have a beneficial effect on the colors of the freeze-dried legume milk powders. Overall, the emulsion capacities of the freeze-dried powders were superior to that of CBMP. The foam capacity and stability of unfermented freeze-dried powders were also superior to the commercial product. The unfermented cowpea powder exhibited by far the greatest foam stability. The results of disc gel electrophoresis revealed that fermentation reduced the amount of soluble protein in the extract. Nitrogen solubility of unfermented freeze-dried powders was similar to nitrogen solubility of CBMP. Fermentation reduced the nitrogen solubility over a pH range 2–10. The viscosities of unfermented and fermented rehydrated freeze-dried powders were clearly superior to that of CBMP. The water adsorption capacities of the unfermented freeze-dried powders were similar to that of the CBMP for both the cowpea and the soybean powders but the freeze-dried peanut milk powder adsorbed less water.

The freeze-dried powders of legume extract studied had functional properties similar or sometimes superior to those of an already commercially available product. Considerable potential exists for incorporating these newly developed products as ingredients in commercial food processing systems.

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Quality Evaluation of Milled Aromatic Rice from India, Thailand, and the United States

R.N. SHARP

ABSTRACT

Five aromatic and two nonaromatic milled rice samples were used to compare the quality of U.S. produced aromatic rice with that of India and Thailand. Jasmine (Thailand) was whiter than all other rice samples tested. Della rice samples (U.S.) were not as white as Basmati (India). Della AR (Arkansas) was less red and less yellow than Della LA (Louisiana). While the uncooked kernels of Della were as long as Basmati or Jasmine, the greater length:width ratio caused Basmati to appear longer than Della. Della and Jasmine were greater than Basmati in 1000 kernel weight. Della samples were classified as having intermediate to high intermediate gelatinization temperature, medium gel consistency, intermediate amylose content and cooking quality, which are characteristic of typical U.S. long grain nonaromatic rice. A sensory panel could not detect a flavor difference between Della AR and either Basmati or Jasmine.

INTRODUCTION

AROMATIC or scented rice is very popular in some parts of the world, but is little known in the U.S. Although aromatic rices are very popular in Pakistan and Northern India, no less than 85 cultivars have been identified in Japan alone (Miyagawa and Nakamura, 1984). Aromatic rice is known by different names according to the geographical area in which it is grown such as Basmati in Pakistan and India and Jasmine in Thailand. Of the various components of aromatic rice quality, aroma is considered to be the most important, and by virtue of their special appeal to consumers, aromatic varieties command a premium in the grain market (Sekhar and Reddy, 1982). Aromatic varieties are said to be low yielding; however, a variety, Della, released by the Louisiana State Experiment Station has improved yields and milling quality (Adair et al., 1973).

Knowledge of the identities of the quality components of rice is important in understanding human perception of rice. The flavor components have received much attention with no less than 100 volatile constituents identified from cooked rice (Yajima et al., 1978), but none of them seem to be related to the nutty or popcorn-like flavor of aromatic rice. In 1982, a potent component, 2-acetyl-1-pyrroline, was identified (Buttery et al., 1982). Odor panelist evaluation described the odor of 2-acetyl-1-pyrroline as "popcorn-like" and ranked different rice varieties in the general order of concentration of this compound (Buttery et al., 1983). Consumer evaluation of rice quality involves more than the odor and flavor. The aromatic type rices exhibit quality characteristics such as grain elongation and volume expansion during cooking, medium amylose content and a low birefringence end point temperature (Juliano, 1979). Furthermore, some varieties in India have been reported to have unusually high lysine, phenylalanine, leucine and methionine contents making them more nutritious (Sekhar and Reddy, 1982).

Aromatic rices are not commonly found in supermarkets in the U.S. but are available in certain shops that specialize in grains and foreign foods. Therefore, the U.S. consumer is not familiar with the quality of aromatic rice nor of the quality differences. This study was conducted to examine some of these quality differences.

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MATERIALS & METHODS

SEVEN SAMPLES representing three aromatic (scented) rice types and two nonaromatic rice samples were used. The aromatic samples were Basmati from India (obtained from two shops in different areas of the U.S.), Della, a U.S. variety (grown in Arkansas (AR) and Louisiana (LA)) and Jasmine from Thailand. The varieties of Jasmine and Basmati were not known. The nonaromatic samples were: Starbonnet, a long grain Arkansas variety and a commercially parboiled sample (Starbonnet variety grown in Arkansas). The Starbonnet and both Della samples were milled with a McGill No. 2 mill according to standard USDA (1976) methods. Other samples were already commercially milled when obtained.

Five replications of milled rice were evaluated to determine Hunter L, a and b color values on "as is basis" using a Gardner Color Difference Meter (CDM), calibrated with a white standard plaque ($L = 92.0$; $a = -1.5$; and $b = 1.0$). Length and width of uncooked and length of cooked grains were determined using a vernier caliper. Volume expansion was determined by measuring the difference in 25°C water displacement of 8.0g of rice before and after cooking. The rice was cooked by placing a wire gauze containing the rice sample into boiling deionized water, then reducing the temperature and allowing the rice to simmer for 20 min. Water uptake at 77°C was determined by the method of Halick and Kelly (1959).

Each rice sample was ground to pass through a 0.25 mm screen using a UDY cyclone mill. Amylose content was determined by the method of Juliano (1971) and alkali spreading value was conducted in accordance with the method of Little et al. (1958). The method of Cagampang et al. (1973) was used to determine gel consistency. Amylographic viscosity measurements of 10% rice flour slurry were obtained by the method of Halick and Kelly (1959) modified by heating to a final temperature of 92.5°C then holding for 15 min before cooling. Pasting temperature was evaluated as the point at which the viscosity reached 10 Brabender Units (B.U.). Total flavors of the cooked aromatic rice samples were compared by 20 panelists using the duo-trio test and statistical significance was evaluated using tables described by Larmond (1977).

The methods of Steel and Torrie (1960) were followed in the calculations of analysis of variance and Duncan's Multiple Range.

RESULTS & DISCUSSIONS

JASMINE was the whitest (highest L value), least red (lowest a value) and least yellow (lowest b value) of the milled rice samples (Table 1). The Basmati samples did not differ and were whiter than the Della samples. However, the Della AR sample was less red and less yellow than either Basmati sample; whereas Della LA was more red than the Basmati samples. As expected, the parboiled sample was darker (lower L value), more red (highest a value) and more yellow (highest b value) than any of the samples. The darker color for parboiled rice could be attributed to both the translocation of pigments from the hull to the endosperm and chemical and physical transformations induced by heating (Luh and Mickus, 1980). For reference, Starbonnet was whiter, but more red and more yellow than Basmati or Della. The degree of milling was not known for the commercially milled samples; therefore, no conclusions can be drawn as to the overall effect of degree of milling. However, these samples appeared to be well milled. Since

Table 1—Tristimulus color values of milled rice kernels

Sample	Hunter color values		
	L	a	b
Basmati I	68.8c[a]	1.7d	16.3c
Basmati II	68.4c	1.7d	16.0d
Della AR	67.2d	0.5e	13.8e
Della LA	67.6d	1.9c	16.0d
Jasmine	72.7a	0.3f	13.2f
Starbonnet	69.8b	2.1b	16.6b
Parboiled	58.6e	3.3a	19.1a

[a] Means within a column followed by the same letter do not differ at the 0.05 level by Duncan's Multiple Range Test.

Table 2—Selected physical measurements of rice kernels[a]

Sample	Length[b] (mm)	Width[b] (mm)	Length:width ratio	1000 Kernel[c] weight (g)
Basmati I	6.93bc[d]	1.80c	3.81b	16.12de
Basmati II	7.21a	1.78c	4.11a	16.69c
Della AR	7.11ab	2.08a	3.45c	18.47ab
Della LA	7.06ab	2.03ab	3.47c	18.70a
Jasmine	7.14ab	1.98b	3.61c	18.29b
Starbonnet	6.58d	—	—	15.89e
Parboiled	6.73cd	—	—	16.30d

[a] Average of three replications.
[b] Twenty kernels per replication
[c] Adjusted to 12% moisture basis.
[d] Means within a column followed by the same letter do not differ at the 0.05 level by Duncan's Multiple Range Test.

Table 3—Measurements of selected parameters used as indices of cooking and eating quality of milled rice[a]

Sample	Alkali spreading value	Gel consistency (mm)	Amylose (%)
Basmati I	6.7a[b]	70b	22.76d
Basmati II	6.7a	56c	24.53a
Della AR	4.1c	45e	22.70e
Della LA	2.9d	56c	23.16c
Jasmine	6.8a	77a	18.20f
Starbonnet	3.1d	51d	22.68e
Parboiled	5.3b	53d	23.49b

[a] Average of three replications.
[b] Means within a column followed by the same letter do not differ at the 0.05 level by Duncan's Multiple Range Test.

Starbonnet and both Della samples were milled under the same conditions, the effect of milling was disregarded.

All the aromatic samples in this study were considered long grain since the length, width ratios were greater than 3.4 regarded by USDA (1976) as appropriate for milled long grain rice. Basmati I did not differ in kernel length from the parboiled sample (Table 2). With that exception all of the aromatic samples were greater in kernel length than the non-aromatic samples. Basmati II had longer kernels than Basmati I while the other aromatic samples were not different from either Basmati sample. Kernels from the Della and Jasmine samples were wider than those from the Basmati. The larger length, width ratios of the Basmati kernels might cause the consumer to judge those samples as having greater length of kernel than Jasmine and Della samples.

The samples differed greatly in alkali spreading value (Table 3). Alkali spreading value is an indicator of gelatinization temperature (Little et al., 1958). Separating the samples according to statistical differences, the samples then would be classified as: Jasmine and Basmati — low; parboiled and Della AR — intermediate; Starbonnet and Della LA — high, intermediate gelatinization temperatures as described by Juliano (1979). Although statistical differences in the gel consistency and amylose percentage were prominent (Table 3), more meaningful expressions might be to group the rice into classifications where a gel consistency of 26 through 40 = hard, 41 through 60 = medium and 61 through 100 = soft gels (Perez, 1979) and amylose percentages of 10 to 20 = low, 21 to 25 = intermediate and more than 25 = high amylose (Juliano, 1979). In this scheme, Jasmine and Basmati I had soft gels and all the other rice samples had medium gels. Jasmine was a low amylose rice while all the other samples were intermediate. Webb et al. (1979) reported that typical U.S. rice varieties followed the pattern: amylose percentage of long grain = 23 to 26 and medium grain = 15 to 20; alkali spreading value of long grain = 3 through 5 and medium grain = 6 through 7. Based on these criteria, Jasmine had cooked qualities similar to U.S. medium grain rice and the Basmati samples showed some of those same tendencies.

Table 4—Comparison of amylographic viscosities of milled rice

Sample	Amylographic viscosity (B.U.)[a]			
	Pasting temp (°C)	Peak	After 15 min at 92.5°C	Cooled to 50°C
Basmatic I	76b[b]	507e	505ab	835bc
Basmatic II	81a	540de	507ab	908a
Della AR	76b	583c	455b	815c
Della LA	80a	630b	545a	865ab
Jasmine	64c	785a	500ab	748d
Starbonnet	80a	553cd	455b	783c
Parboiled	80a	150f	150c	258e

[a] B.U. = Brabender Units. Average of duplicate measurements.
[b] Means within a column followed by the same letter do not differ at the 0.05 level by Duncan's Multiple Range Test.

Table 5—Evaluation of cooking quality of milled rice[a]

Sample	Length of[b] cooked kernel (mm)	Expansion[b] ratio	Volume expansion during cooking	Water uptake at 77°C (mL)
Basmati I	13.67a[c]	1.95a	5.2ab	119.1c
Basmati II	14.05a	1.97a	5.0b	117.3c
Della AR	9.68bc	1.36b	4.7c	80.0d
Della LA	10.03b	1.42b	5.0b	73.8d
Jasmine	9.91bc	1.39b	5.3a	330.4a
Starbonnet	8.86d	1.37b	4.6c	75.1d
Parboiled	9.17cd	1.37b	4.1d	209.2b

[a] Average of three replications.
[b] Twenty kernels per replication.
[c] Means within a column followed by the same letter do not differ at the 0.05 level by Duncan's Multiple Range Test.

Pasting temperature was taken as the temperature at which the amylographic viscosity measured 10 B.U. and may be used to approximate gelatinization temperature by subtracting 3°C (Juliano et al., 1964). Basmati II, Della LA, Starbonnet and parboiled did not differ and had higher pasting temperatures than Basmati I, Della AR and Jasmine (Table 4). The viscosity patterns differed considerably between the samples (Table 4). Jasmine developed the highest peak viscosity but the hot paste viscosity (holding at 92.5°C for 15 min) was only different from the parboiled sample and after cooling to 50°C was lower than all except parboiled. Della AR exhibited viscosity values more nearly like Starbonnet than did any of the other samples.

An examination of the length of cooked kernel (Table 5) showed that the Basmati samples did not differ and had longer cooked kernel length than the other samples. The length of cooked kernel of both Della and Jasmine were not different. All the aromatic types had greater length of cooked kernel than Starbonnet; however, Jasmine and both Della samples did not differ from either Starbonnet or parboiled in expansion ratio. Both length of cooked kernel and expansion ratio are important quality considerations (Juliano, 1979). By these criteria the Basmati samples would have better cooking quality than Della and Jasmine. Volume expansion during cooking or cooking yield indicated that Jasmine had total expansion equal to Basmati I and greater than the other samples. With the exception of Della AR, all aromatic samples exhibited greater expansion during cooking than Starbonnet. The water uptake at 77°C distinctly separated the milled samples into separate groups relating to cooking behavior.

The sensory panel was unable to distinguish between the Basmati I, Basmati II, Della AR and Jasmine, and between

Table 6—Results of duo-trio sensory evaluation

Test samples		Correct responses[a]
Basmati I	VS Basmati II	10
Basmati I	VS Della AR	9
Basmati I	VS Della LA	16
Basmati I	VS Jasmine	15
Basmati II	VS Della AR	12
Basmati II	VS Della L.A	15
Basmati II	VS Jasmine	14
Della AR	VS Della LA	13
Della AR	VS Jasmine	13
Della LA	VS Jasmine	14

[a] Number of panelists correctly identifying the unknown sample. N = 18. Fifteen correct responses required for significance at the 0.05 level.

the Della samples (Table 6). Although the panel was unable to detect a difference between specific samples, there was indecision as to which had the more intense flavor. The Della samples were examples of this situation. The lack of differences between Della AR and either Basmati I, Basmati II or Jasmine indicated that Della AR tended to be more aromatic than Della LA. The aromatic types were not tested against the non-aromatic types for flavor difference.

CONCLUSIONS

ASSUMING that all samples had comparable degree of milling, Jasmine was less red, less yellow and more white than all other samples and was the only sample more white than Starbonnet. Della AR was less red and less yellow than Della LA or either Basmati samples. Grains of the U.S. variety, Della, were as long as grains from the other countries; however, the greater kernel width gave Della a higher 1000 kernel weight than Basmati. The overall cooking quality of Della was more like that of Starbonnet than was Basmati or Jasmine. Between the U.S. grown aromatics, Della AR tended to have cooking quality more like non-aromatic rice than did Della LA; however, sensory panelists judged Della AR to have flavor qualities more like the India and Thailand grown aromatic rice than did Della LA.

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Effects of Lysine and Methionine Fortification on Dough and Bread Characteristics

MARIA LUZ FERNANDEZ DE TONELLA and MARIA SUSANA YEPIZ

ABSTRACT

Wheat flour from the Hermosillo Hard Red Spring cultivar in Sonora, Mexico was fortified with various levels of lysine and methionine. The effects of added amino acids on the rheological properties were measured by the Brabender Farinograph and the Chopin Alveograph. Nutritional characteristics were measured by Protein Efficiency Ratio (PER). Dough development and stability times were not significantly different between control and fortified flours. Water absorption and Alveograph results indicated differences between samples. PERs were significantly improved by fortification. Overall bread acceptability was not significantly different from unfortified samples.

INTRODUCTION

WHEAT is an important cereal because it can be used for the preparation of many products. Bread, the major wheat-based food product, is one of the least expensive, most important staples in the world. Lysine is the first limiting amino acid in wheat flour. Tryptophan, threonine and methionine are also low when compared to the FAO standard (FAO, 1973). Fortification with essential amino acids has become a common method used to improve the nutritional quality of wheat products (Khoeler, 1964; Kent, 1984).

Several attempts have been made to improve the nutritional value of bread by the addition of lysine and other essential amino acids. Lysine added to bread at the same concentration as found in 6% nonfat dry milk (0.166%) increases its PER from 1.49 to 1.99 (Sabistan and Kennedy, 1957). Supplementation of breads with 3.3% lysine caused marked increases in protein quality (Fleming and Sosulski, 1977). PER values varied from 1.04 in the control to 2.08 in the lysine supplemented bread. Other studies on bread fortification have been reported with lysine, methionine and threonine the major limiting amino acids in wheat (Arafah et al., 1980). Important increases in PER values were observed when these amino acids were added.

Studies have been made on the influence of lysine and methionine fortification on physical properties of tortillas and other corn-based food products (Tonella et al., 1983); however, very little published information on the influence of these essential amino acids on rheological properties of wheat-based dough is available.

The purpose of this study was to fortify wheat flour with various levels of lysine and methionine in order to determine the influence of these added amino acids on rheological properties of dough and on nutritional improvement and sensory attributes of the breads. These two amino acids were chosen because of their economic availability. In addition, lysine is the first limiting amino acid in wheat flour (Hepburn et al., 1966), and methionine has a significant effect on flavor.

MATERIALS & METHODS

Materials

Hermosillo cultivar, hard red spring wheat from 1981 crop was used. Lysine and methionine were from Cia. Albamex, Mexico city.

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Table 1—Flour blends with added amino acids

Blend	Amino acids (g/100g flour)	
	Lysine	Methionine
Control	—	—
1	0.8	—
2	1.6	—
3	3.2	—
4	—	0.125
5	—	0.250
6	—	0.500
7	0.8	0.125
8	1.6	0.250
9	3.2	0.500

Flour preparation

Wheat was separated from extraneous matter by selection on basis of size in a Brabender Labofix (Duisberg, Germany) and divided into 2.5 kg aliquots. During 24 hr prior to milling on a Brabender Quadrumat Senior (Duisberg, Germany), the material was equilibrated at room temperature with distilled water at 15% moisture. An extraction rate of 70% was obtained.

Preparation of fortified flours

Flour was mixed with the desired amino acid at various concentrations (Table 1).

Chemical analysis

Protein, fat, ash and moisture were determined by AACC methods (AACC, 1976).

Amino acid analysis

Lysine and methionine were determined in the control and fortified flour (sample 8, Table 1) and fortified bread by microbiological methods. *Leuconostoc mesenteroides* was used according to Mattern et al., (1968). Standard curves were established for each assay with tubes containing 6 levels of the amino acids. The L form of the amino acids was used for preparation of standard solutions. Samples were acid hydrolysed, neutralized, filtered, diluted and then added to incubation tubes. After incubation for 72 hr at 37°C, the lactic acid produced was titrated with 0.05N NaOH.

Rheological determinations

Farinograms were made by the Constant Flour Weight Method using 300g sample with a Brabender Farinograph (AACC, 1976; method 54.21). Water absorption, development and stability times were measured in all flours and compared to the control. Other parameters such as maximum pressure or resistance to stretching (P), extensibility of dough (G) and deformation work (W) were measured by the Chopin Alveograph (Paris, France) using the standard method (Chopin, 1978).

Bread baking procedure

Bread was prepared on percent flour basis from the following formula: flour 100%, sugar 3.0%, shortening 1.3%, salt 0.7% and yeast 2%. Yeast was activated for 15 min with 100 mL of water at 30°C. The rest of the ingredients were slowly mixed in a Hobart mixer with 100 mL of water. Yeast was added slowly and mixing continued until dough was not sticky. It was then fermented for 1 hr at 30°C, punched and divided into 200g pup loaves. After another 30 min, loaves were molded and baked at 250°C for 25 min.

Nutritional quality

Nutritional quality was determined on the control and fortified bread as protein efficiency ratio (PER) using a modification of the AOAC method (AOAC, 1980) with Sprague Dawley rats. The modification

Table 2—Experimental diet formulations

Ingredient	Diet		
	%		
	Casein[a]	Bread[b]	Fortified bread[c]
Casein	9.75	—	—
Bread	—	74.28	—
Fortified Bread	—	—	72.86
Vitamins	1.0	1.0	1.0
Minerals	5.0	5.0	5.0
Fiber	1.0	1.0	1.0
Fat	8.0	8.0	8.0
Glucose	2.0	2.0	2.0
Starch	73.25	2.0	2.0

[a] Protein - 82%, based on 8% Kjeldhal protein
[b] Protein - 11%, based on 8.16% Kjeldhal protein
[c] Protein - 10.8%, based on 7.85% Kjeldhal protein

Table 3—Proximate composition[a]

Component	Flour	Bread	Fortified bread[b]
		%	
Moisture	13.49	4.37	4.96
Protein	11.62	10.77	10.98
Fat	1.17	3.65	4.26
Ash	0.54	1.67	1.73
Carbohydrates[c]	73.18	79.54	78.07

[a] Dry weight basis
[b] 1.6% lysine and 0.25% methionine (blend #8)
[c] Calculated by difference

consisted of using approximately 8% protein in the diet instead of 10% (Table 2). Protein must be adjusted in the case of many cereals because it is impossible to achieve 10% protein for experimental diets unless the cereal is fortified. To solve this problem, a protein content lower than that established in the official method must be used. The alternative would be the utilization of mice since their protein requirements are lower (Cossack and Webber, 1983). Each diet contained approximately 8% protein (as Kjeldhal nitrogen). Levels of 1.6% lysine and 0.25% methionine were chosen for fortification (Table 1). Higher levels of amino acids were not selected, since in preliminary sensory evaluations, a detectable flavor resulted with higher methionine fortification.

Sensory evaluation

A 24 member untrained panel of faculty, staff and students evaluated the breads for the sensory attributes of texture, flavor, odor and color on a 1 to 10 hedonic scale with 10 = like extremely, 5 = neither like nor dislike and 1 = dislike extremely. Four samples were presented to the panelists at each session in random order. The sensory evaluation tests were performed in a comfortable, clean environment. Tests were done in replicate. Samples were evaluated within approximately 10 min after being reheated at 30°C for 5 min. Bread had been baked the day before. Sensory analysis data were analyzed to assess significant differences between hedonic characteristics of samples using ANOVA.

RESULTS & DISCUSSION

Proximate analysis

Proximate analysis of wheat flour (control) and the breads were in the expected ranges (Table 3). Protein values for wheat, though, were slightly low compared to other reported values (Dekeman et al., 1982). Protein values for bread and fortified bread, 10.77% and 10.98% respectively, were in the expected range according to formulation of the breads. Fat values ranged from 1.17% in flour to 4.26% in fortified bread. Values for fat in bread were higher due to added shortening. Ash and carbohydrates also increased in breads due to the addition of salt and sugar.

Amino acid analysis

Lysine was determined in the control and in the fortified bread (sample # 8, Table 1) to assess possible lysine losses during bread making (Table 4). Methionine was only determined in control. Lysine in the fortified bread was low compared to the FAO pattern (FAO, 1973) (Table 4). Sensory attributes, particularly flavor and odor, limited the use of higher concentrations of amino acids, especially methionine. A 10% loss of lysine was observed after baking. This percentage was in the expected range (Saab et al., 1981). Methionine in fortified bread could not be determined because the added amino acid was always destroyed by acid hydrolysis (Yepiz and Fernandez de Tonella, unpublished data).

Table 4—Lysine and Methionine in Flour and Bread Compared to the FAO pattern

Amino acid	Material			
	Control	Fortified flour[a]	Fortified bread	FAO
		g/100g protein		
Lysine	2.8	4.4[b]	4.0	5.5
Methionine	1.4	1.65[b]	—	2.2

[a] From sample #8
[b] The sum of the respective amino acid contents in the control and blend #8.

Table 5—Farinograph parameters in control and fortified flours

Sample	Time		
	Dough development	Stability	Water absorption (%)
	min		
Control	6.8	14.0	59.7
1	7.1	12.9	59.8
2	6.8	12.5	59.3
3	7.3	12.7	59.2
4	7.3	10.7	62.6[a]
5	7.3	9.9	62.4[a]
6	7.5	8.9	62.9[a]
7	7.3	12.3	61.5[a]
8	7.5	11.3	61.5[a]
9	7.3	11.8	59.9

[a] Statistically different from control, (p = 0.05) as determined by Newman-Keules procedure (Rosner, 1982).

Table 6—ANOVA F values for Farinograph tests on flours

Parameter	Fortification		
	Lysine[a]	Methionine[b]	Lys. + Met.[c]
Dough development	4.03	1.73	2.22
Stability	2.6	3.73	1.16
Absorption	3.8	140.21[d]	93.87[d]

[a] Blends # 1, 2 and 3 compared to control
[b] Blends # 4, 5 and 6 compared to control
[c] Blends # 7, 8 and 9 compared to control
[d] Statistically significant at p = 0.05

Rheological determinations

Water absorption, development and stability times were the chosen parameters for comparison in the Farinograph determinations (Table 5). This selection was made because these parameters are the most representative of rheological properties of wheat dough and bread (Schlesinger, 1957). Also water absorption appears to be an essential characteristic for flour (Sollars and Rubenthaler, 1975).

Analysis of variance (ANOVA) was performed to the farinograph results (F values, $p = 0.05$) (Table 6) to compare every added amino acid to the control.

The farinograms were typical for this type of wheat. Farinograms are used to give a general profile of the mixing behavior of the dough. A good correlation has been observed between general strength of a flour and its protein content (Shuey, 1972). Flours with low protein have shorter dough development times (peak time) while those with higher protein values give longer dough development times. Markley et al. (1939) found a correlation coefficient of 0.88 between the peak times and crude protein of a series of 89 wheat samples. None of the fortified flours had dough development times that were significantly different from the control (Table 6).

An effect of decreasing mixing time and therefore stability

Table 7—Alveograph parameters in control and fortified flours

Sample	Parameter		
	Stretching P(mm)	Elasticity G(ml)	Work (x10-4joules)
Control	123.9[a]	21.3[a]	322.2[a]
1	131.6[b]	21.3[a]	339.3[b]
2	126.3[a]	21.7[a]	343.2[b]
3	122.4[a]	21.9[a]	345.1[b]
4	144.7[b]	20.6[b]	380.5[c]
5	131.8[b]	21.9[a]	365.7[c]
6	145.7[b]	20.5[b]	377.3[c]
7	145.5[b]	21.1[a]	391.1[c]
8	141.9[b]	20.8[a]	360.3[c]
9	131.9[b]	21.5[a]	365.4[c]

[a-c] Values having different superscripts are statistically different from control and from each other (p=0.05) as determined by Newman Keules procedure (Rosner, 1982).

Table 8—Protein efficiency ratio of control and fortified bread

Diet	PER
Casein	2.5±0.30[a]
Fortified Bread	1.8±0.17[b]
Bread	1.3±1.15[c]

[a-c] Values having different letters are statistically different (p < 0.05).

Table 9—Sensory evaluation scores for control and fortified breads

Attributes	Bread Product			
	Control	1.6%lys	0.25%meth.	1.6%lys +0.25meth
Color	8.4	8.6	9.0	8.8
Odor	7.7[a]	8.3[a]	7.8[a]	8.9[b]
Flavor	7.9	8.2	8.3	8.3
Texture	7.7	7.6	8.2	8.2

[a,b] Values having different letters are statistically significant (p=0.05)

Table 10—Analysis of variance for bread sensory scores

Attribute	Source	SS	df	MS	F[a]
Color	Product	3.12	3	1.04	1.25
	Error	76.17	92	0.83	
Odor	Product	21.78	3	7.26	4.0[b]
	Error	167.03	92	1.82	
Flavor	Product	10.08	3	3.36	1.8
	Error	166.52	92	1.81	
Texture	Product	7.38	3	2.46	1.77
	Error	127.51	92	1.38	

[a] F value = 2.71 at p = 0.05
[b] Statistically significant

was noticed when water soluble extracted gliadins were added to dough (Preston et al., 1975). Mixing time decreased even more when these gliadins were enzymatically hydrolyzed and added to the dough. Stability time values were not significantly different in any of the fortified flours compared to control (Table 6).

Absorption is a measurement of water requirements of flour with its limitations. An increase in absorption has been observed with increases in protein (Merrit and Stombery, 1941). Except for sample #9, absorption increased when methionine or the mixture of amino acids was added (Table 5). This increase was highly significant in the case of added methionine (Table 6). This effect is somewhat difficult to explain. Possibly some of the added lysine interacted with wheat protein by formation of polar bonds and, therefore, the overall effect on water absorption remained the same as in the case when only this amino acid was added. On the other hand, it is believed that since methionine is a hydrophobic amino acid (Lehninger, 1982), it may repel water and wheat protein polar bonds and, therefore, permit a higher interaction between the two. As a consequence, an increase in water absorption resulted. When the mixture of amino acids was added, this same effect prevailed though a small decrease in this parameter was observed when lysine and methionine acted together.

Maximum stretching (P), dough extensibility (G), and deformation work (W) were the three parameters evaluated by the Chopin Alveograph (Table 7) with ANOVA based on a one way test for F values (p = 0.05) (Table 8).

Alveographic results showed that lysine had a peculiar effect on maximum stretching (P) of dough. At low concentrations there was an increase in P (Table 7) while at higher concentrations there was no significant difference from the control (Table 7). The Newman-Keules procedures for testing differences between means (Rosner, 1982) showed that the 0.8% lysine fortified flour was significantly different from the other three (p=0.05). Methionine alone and the amino acid mixture showed statistical significance on this parameter compared to control. (Table 7). Lysine at lower concentrations seemed to react with the fraction of gluten that was responsible for stretching of dough with a resultant increase in P value. At higher concentrations, it might interact with itself, as a decrease in this parameter was always observed.

Added methionine also gave a significant increase in elasticity (Table 7). Values for this parameter remained the same when either lysine or the mixture of amino acids was added. Therefore, methionine probably had an effect on glutenin, the only constituent of flour that exhibits significant elasticity when isolated (Greenwood and Ewart, 1974).

Deformation work (W) changed significantly with all fortifications (Table 7). This parameter was influenced by both P and G so that as P was affected in all cases, W followed the same pattern.

Nutritional evaluation

An increase of 38.6% in PER values was observed in fortified bread compared to the control (Table 8). It is believed that no higher increase in supplemented bread was found due to the effect of methionine fortification. Higher amino acid levels were not used because methionine gave an undesirable flavor to baked products. In addition, the second limiting amino acid in bread is tryptophan (Bressani et al., 1960). In this case, supplementation was limited to the above mentioned amino acids because of cost. Methionine fortification might have somehow made tryptophan even more limiting. In spite of these drawbacks, an important increase in PER was found.

Sensory attributes

Sensory evaluation indicated a high acceptability for fortified breads (Table 9). This test was performed on breads from flours #2, 5, and 8 (Table 1). Hedonic characteristics were the same for all breads except in case of odor where there was a significant difference (p=0.05) among samples (Table 10).After comparing means by the Newman-Keuls procedures, it was concluded that the difference was due to bread from sample #8. These two amino acids together gave breads a characteristics odor which made them highly acceptable.

Methionine appeared to have a greater effect than lysine on rheological properties of dough. It caused an increase in water absorption as well as in tenacity and elasticity. When both lysine and methionine were added, these same effects were produced although to a lesser degree than with methionine alone. These characteristics seemed to be quite acceptable for the baking process. In addition, when lower concentrations of amino acids were used as was the case in bread baking, acceptability was improved as compared to the nonfortified bread. Furthermore, an important PER increase was observed. Therefore, it was possible to obtain good rheological characteristics for dough as well as improved nutritional and sensory characteristics for bread with lysine and methionine fortification.

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Use of Vegetable Oils at Reduced Levels in Cake, Pie Crust, Cookies, and Muffins

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ABSTRACT

Sensory acceptability and physical characteristics of yellow cake, pie crust, drop sugar cookies and muffins prepared with reduced levels of oils (soybean and safflower) of intermediate and high polyunsaturated/saturated fatty acid (P/S) ratio and a standard level of hydrogenated vegetable shortening were evaluated. Both types and all levels of oil compared satisfactorily with vegetable shortening in both cake and pie crust for all sensory characteristics judged. Drop sugar cookies made from oils were similar in flavor to those made with shortening, but were less liked for appearance, crispness and overall preference. Muffins prepared with the oils were preferred over the shortening for appearance but there were no definite trends in preference for other characteristics.

INTRODUCTION

VEGETABLE OILS are generally excellent sources of polyunsaturated fatty acids. Current dietary goals for the United States recommend that one-third of the total fat in the diet should be polyunsaturated. Additionally, reduction of overall fat consumption to about 30% of food energy was recommended by the Food and Nutrition Board (1980). Prior to this recommendation, U.S. per capita consumption of edible vegetable oils and shortening increased by 66% and 34%, respectively from 1965 to 1979, while animal source fats decreased; consumption of total fats increased 21% during that same period (Chung and Pomeranz, 1985).

Although general consumption of fats and oils has increased, recent trends in the baking industry are to reduce total usage of fats and oils and to replace plastic fats with liquid vegetable oils (Chung and Pomeranz, 1983; Harnett and Thalheimer, 1979a). Two of the changes recommended in a recent study by Salz et al. (1982) were replacement of most high fat baked products by those lower in fat and replacement of some animal fat by polyunsaturated vegetable oil products.

Several studies have been reported in the literature on the performance of vegetable oils in pastry and cake. Matthews and Dawson (1963) found vegetable oils to be good shortening agents in pastry. Dreher et al. (1983) reported that vegetable oils produced pie crusts with better flavor and flakiness than partially hydrogenated vegetable shortening. The effects of both type and level of shortening on batter viscosity, specific gravity and shear force values of white cakes were studied by Matthews and Dawson (1966). As the level of shortening increased, specific gravity and shear force values decreased, while batter viscosity increased with increased fat level up to 50%. Harnett and Thalheimer (1979b) replaced plastic fat in cake with vegetable oils and reduced total fat. Subjective and objective testing showed that a 33% and 60% decrease in total fat in two cake formulas provided best texture characteristics. They concluded that with a proper emulsifier system, oil can be an effective alternative to plastic fats in bread, sweet goods and cakes. Paton et al. (1981) studied the effect of an oil mixture at 0, 50, 100, and 150% of normal levels on development of cake structure. As the level of oil mixture was increased, maximum cohesive force was reduced, indicating a tenderizing role for liquid shortening ingredients. Little research was found in the recent literature regarding the performance of vegetable oils in cookies or muffins.

The purpose of this study was to determine the acceptability and physical characteristics of yellow cake, pie crust, drop sugar cookies and muffins prepared with reduced levels of soybean and safflower oils and a standard level of hydrogenated vegetable shortening.

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MATERIALS & METHODS

Experimental design

The two vegetable oils studied were commercially available safflower and soybean oils with polyunsaturated/saturated fatty acid (P/S) ratios of 8.2 and 4.0, respectively. The control vegetable shortening (Crisco) was composed of partially hydrogenated soybean and palm oils with mono- and diglycerides added and had a P/S ratio of 0.5. The P/S ratios were calculated from data included in Reeves and Weihrauch (1979). The standard level of vegetable shortening was used in four products: a commercial yellow cake mix, pie crust, drop sugar cookies, and muffins. Three reduced levels of both oils were used in the cake mix and muffins. Two reduced levels of both oils were used in the pie crust and drop sugar cookies.

Preparation of samples

Cake. Cake was prepared using a formula consisting of a commercial national brand yellow cake mix, 223g water, 155g whole egg and one of the following: 71g vegetable shortening; 53, 36 or 24g safflower or soybean oil. The cake batter was mixed using a Kitchen Aid mixer (Hobart Manuf. Co., Troy, OH) on slow speed for 2.5 min. The cake batter was placed in a 23 × 33 cm loaf pan which had been coated with non-stick spray and was baked for 30 min at 177°C. Cake was allowed to cool on a rack for 1 hr before wrapping and freezing (−22°C) for up to 1 wk prior to sensory evaluation.

Pie crust. Each pie crust formulation consisted of 130.0g sifted all purpose flour, 33.4g cold water (20°C) and one of the following: 65g vegetable shortening; 54 or 43g safflower or soybean oil. Solid shortening was cut into the flour with a pastry blender until pea-sized. The oils were mixed with flour prior to incorporation of water. The crust was rolled between sheets of plastic wrap using rod guides to obtain a uniform thickness (2 mm). The crusts were cut in 7.62 × 2.54 cm strips and baked at 220°C for 10 min. Pie crusts were cooled for 30 min, wrapped and frozen for a maximum of one week prior to sensory evaluation.

Drop sugar cookies. Cookies were prepared using a formulation which consisted of 75g all purpose flour, 1.15g sodium bicarbonate, 55g white sugar, 15g brown sugar, 1.15g salt, 25g beaten whole egg, 2 mL vanilla and one of the following: 55g vegetable shortening; 40 or 34g safflower or soybean oil. The oil and sugars were mixed for 90 sec using a Kitchen Aid mixer on slow mix. After addition of egg and vanilla, the dough was mixed another 90 sec. The dry ingredients were blended, added and beaten for 60 sec. A No. 70 scoop was used to measure and drop cookies onto ungreased trays. These were baked at 190°C for 10.5 min. Cookies were allowed to cool for 30 min before double wrapping and freezing for a maximum of one week prior to sensory evaluation.

Muffins. The muffin formulation consisted of 268g all purpose flour, 100g sugar, 12.75g baking powder, 3.26g salt, 43g beaten whole egg and one of the following mixtures: 53g vegetable shortening

and 185 mL whole milk; 36g safflower or soybean oil and 200 mL milk; 27g of either oil and 210 mL milk; or 14g of either oil and 225 mL milk. The dry ingredients were blended in a mixing bowl. The egg, milk and shortening/oil were combined and added all at once to dry ingredients. The batter was mixed only until moistened. Batter (42g) was dipped with a No. 30 scoop (Campbell et al. 1979) into muffin cups which had been coated with nonstick spray. All muffins were baked at 205°C for 18 min, cooled for 30 min, wrapped and frozen for up to one week prior to sensory evaluation.

Sensory evaluation

The consumer sensory panel for each product consisted of 75 untrained members who were paid participants in this study. Panelists included students, faculty and staff from North Dakota State University who responded to advertisements displayed on the campus.

Sensory quality attributes were evaluated using a 9-point hedonic rating scale (1 = dislike extremely to 9 = like extremely) (Amerine et al., 1965; Johnston, 1979). Yellow cake was evaluated for appearance, moistness, flavor and overall preference. Attributes of color, flavor, texture and overall preference were considered for pie crust. Cookies were judged on appearance, crispness, flavor, chewiness and overall preference. Muffins were evaluated for appearance, flavor, mouthfeel, texture, and overall preference.

Sensory evaluation sessions for each product were conducted on separate days from mid-morning to mid-afternoon. The tests were performed in partitioned booths with overhead fluorescent lighting. Samples were coded with three-digit random numbers and presented sequentially in a balanced order which differed among individual panel members. Individual scorecards were presented with each sample and instructions for marking scorecards were posted in each judging booth. Panelists were supplied with water (22°C) for mouth rinsing between samples.

Analysis of data

Prior to statistical analysis, descriptors were converted to numbers (1 = dislike extremely to 9 = like extremely). The General Linear Model (GLM) procedure of the Statistical Analysis System (SAS) computer package was used to perform statistical analysis. Differences in preference for each characteristic of each baked product due to varying type and level of fat were tested for significance using analysis of variance techniques (both parametric and nonparametric). Duncan's Multiple Range Test was used as post hoc procedure when the analysis of variance indicated significant differences in means. A level of significance of $p \leq 0.05$ is used throughout the analysis unless otherwise noted. Descriptive statistics are reported with small sample size for some objective analyses.

Objective evaluation

All four products were evaluated for color which was determined on a Gardner Tristimulus XL 800 Colorimeter with an XL 845 Circumferential Sensor using the L, a, b scale compared to a white standard (XL-23-246-D). Kcalories were calculated from data included in Adams (1975). Volume of cake and muffins was measured by rapeseed displacement in a volumeter. Cake batter viscosity was determined after mixing and prior to baking with a Brookfield synchroelectric viscometer using Spindle RV-6, 20 rpm at ambient temperature. Flakiness was determined by measuring the height of each of two rectangles of baked pastry at the centerpoint of each of the four sides of the samples and then averaging the eight values obtained (McWilliams, 1977). Pie crust shrinkage was determined by the following formula:

$$\% \text{ Shrinkage} = \frac{\text{Area before baking - Area after baking}}{\text{Area before baking}} \times 100$$

Cookie spread was determined by the following formula:

$$\% \text{ Spread} = \frac{\text{Diameter after baking - Diameter before baking}}{\text{Diameter before baking}} \times 100$$

RESULTS & DISCUSSION

Quality of cake

Sensory and objective results for cake are shown in Table 1. The average sensory scores of the seven cake treatments did not differ significantly for any of the characteristics measured. Judges could not detect significant differences between cake treatments (various levels and types of oil) for any sensory characteristic. There was variation in Gardner color values for the cakes made with different types and levels of fat. However, panelists did not perceive the Gardner color variability as a negative factor as sensory scores for appearance were not significantly different. No trend in batter viscosity or volume of cake was observed with varying level or type of fat used. Kcalories decreased when less oil was used. When safflower and soybean oil at all three reduced levels were used in a commercial cake mix, those cakes were as acceptable as those made with vegetable shortening.

Pie crust characteristics

The pie crusts were cut into 7.62 × 2.54 cm pieces to insure that judges would have uniform samples for evaluation. Also it was more accurate to determine flakiness and shrinkage of the pieces rather than the whole crust. The pie crust characteristics are shown in Table 2. Significant differences were not found between the average sensory scores of the five pie crust treatments. Judges could not detect differences between treatments (various types and levels of fat) for any sensory characteristic. There was slight variation between Gardner color values for L, a and b for the top and bottom of the crusts. The type or level of fat had little or no effect on pie crust color. The flakiness of the crusts ranged from 4.25–5.08 mm, with the 54g safflower oil crust being the least flaky and the vegetable shortening crust the most flaky. The 43g safflower crust was most similar in flakiness to the vegetable shortening crust. However, these differences do not correspond to texture scores as all texture scores were not significantly different. The pie crusts made with soybean oil shrank the least (17.5 and 18.0%) while the shrinkage for the safflower oil crusts was somewhat greater (19.3 and 19.6%). The difference in the range of shrinkage was 2.1%. As expected, kcalories decreased when oil was reduced. Substitution of soybean and safflower oils at reduced levels in this pie crust formula yielded as acceptable crusts as the vegetable shortening.

Drop sugar cookies

Data for drop sugar cookies are shown in Table 3. Highly significant differences were found between the treatments on average appearance and crispness sensory scores ($p = 0.0001$ and $p = 0.0003$, respectively). That is, the judges did indicate preferences of oil type and level in sugar cookies based on the appearance and crispness of the product. Average overall preference and chewiness sensory scores of the five treatments were significantly different at the 0.05 level. There was a significant difference in the preferences of the judges for varying oil type and level of the chewiness and overall preference of the drop sugar cookies. The judges did not perceive significant differences in the flavor of the drop sugar cookies made with varying oil types or levels.

Duncan's Multiple Range Test (Table 3) was used to identify differences between treatments for the cookie characteristics for which significance was found. Flavor differences were not significant, therefore Duncan's Multiple Range Test was not applied to flavor. Cookies made with vegetable shortening were judged to be best in appearance. The cookies with the two levels of safflower oil and 34g of soybean oil were judged similar and the least liked. Cookies made with vegetable shortening and 40g soybean oil were judged best for crispness. The safflower and soybean oil treatments of 40g were judged similar. Additionally, the 34g treatment of soybean oil and both levels of safflower oil were judged similar and least preferred for crispness. For chewiness, cookies made with vegetable shortening, 34 and 40g soybean oil and 40g safflower oil were judged the best, and similar. The cookies with 34 g of both safflower and soybean oil were judged similar. Vegetable

Table 1—Yellow cake characteristics

Cake		Color L	Color a	Color b	Batter viscosity[a] (CPS)	Volume[b] (ml)	Kcalories[c]	Sensory scores[d] Appearance	Moistness	Flavor	Overall preference
n		1	1	1	1	1	1	75	75	75	75
Safflower oil											
53g	top	39.74	14.81	17.81	17,750	2,316	247	6.79	6.96	6.95	6.70
	inside	73.18	1.20	31.24							
36g	top	45.38	14.52	21.53	13,250	2,412	234	7.14	7.01	7.01	6.97
	inside	76.62	1.34	33.88							
24g	top	51.77	15.23	19.77	13,125	2,496	225	6.88	7.12	6.87	6.81
	inside	73.71	1.16	32.80							
Soybean oil											
53g	top	43.09	15.83	20.76	16,750	2,364	247	7.12	7.48	7.18	7.26
	inside	73.32	1.36	33.38							
36g	top	38.33	13.94	16.52	17,250	2,400	234	7.03	6.84	6.84	6.83
	inside	73.54	1.32	33.43							
24g	top	44.06	14.07	20.60	23,250	2,304	225	7.05	6.78	6.87	6.81
	inside	73.71	0.89	31.77							
Vegetable shortening											
71g	top	39.09	12.27	16.27	25,250	2,352	260	7.32	6.78	6.87	6.75
	inside	75.71	0.83	33.22							
F Value:								1.29	1.65	0.45	1.22

[a] 25°C
[b] 9″ × 13″ cake
[c] 3″ square (1/12 cake)
[d] Hedonic ratings: 1 = dislike extremely; 9 = like extremely

Table 2—Pie crust characteristics

Crust		Color L	Color a	Color b	Flakiness (mm)	Shrinkage (%)	Kcalories[a]	Sensory scores[b] Color	Flavor	Texture	Overall preference
n		1	1	1	12	4	1	75	75	75	75
Safflower oil											
54g	top	69.90	0.86	20.26	4.25 ± 0.67	19.6 ± 2.87	894	6.11	4.75	5.58	5.16
	bottom	71.80	−0.01	19.39							
43g	top	67.97	1.37	20.23	5.00 ± 0.60	19.3 ± 3.77	799	6.33	5.20	5.96	5.47
	bottom	70.89	0.09	19.20							
Soybean oil											
54g	top	70.11	0.71	18.88	4.75 ± 0.45	18.0 ± 1.63	894	6.39	5.52	6.46	5.74
	bottom	70.70	0.23	19.62							
43g	top	69.18	0.80	19.09	4.58 ± 0.67	17.5 ± 4.26	799	6.31	5.20	5.85	5.41
	bottom	70.49	0.19	19.49							
Vegetable shortening											
65g	top	69.12	1.51	20.77	5.08 ± 0.90	18.8 ± 3.14	989	6.43	5.61	6.43	5.82
	bottom	71.84	0.43	19.40							
F Value:								0.52	2.41	2.19	1.65

[a] per total recipe
[b] Hedonic Rating: 1 = dislike extremely; 9 = like extremely

shortening cookies were judged the best for overall preference; all oil treatments were similar.

There was some variation in Gardner color values, although most scores for each value were similar, indicating that type and level of fat had little or no effect on the color of the various cookies. The percentage of spread was similar and ranged from 79.35–83.00%. Kcalories of cookies decreased with the reduced oil treatments. Although judges detected no differences in flavor of cookies made with any type or level of fat, drop sugar cookies made with vegetable shortening were the most acceptable for appearance, crispness and overall preference.

Muffin quality

Results for muffins are shown in Table 4. The analysis of variance test found all muffin characteristics significantly different ($p < 0.05$). Appearance, flavor, mouthfeel and overall preference differences were significant ($p < 0.01$). There was a significant difference in the preference of the judges for varying oil type and level in the muffin for all sensory characteristics tested.

Differences between treatments for muffin characteristics were identified by Duncan's Multiple Range test and are shown in Table 4. All safflower and soybean oil treatments (levels) were judged similar for muffin appearance, while muffins made with vegetable shortening (also the highest level of fat) were least preferred ($p < 0.05$) for appearance. Muffins made with vegetable shortening, 14 and 36g soybean and 36 g safflower oils were judged similar, and best for flavor. There was no difference in preference ($p > 0.05$) of flavor of muffins made with

Table 3—Drop sugar cookie characteristics

Cookie		Color L	Color a	Color b	Spread %	Kcalories[a]	Sensory scores[b,c] Appearance	Crispness	Flavor	Chewiness	Overall preference
n		2	2	2	18	1	75	75	75	75	75
Safflower oil											
40g	top	46.96	8.93	17.90	80.33 ± 3.89	77	6.04^{z}	6.31^{yz}	6.43	6.26^{x}	6.19^{xy}
	bottom	46.46	10.80	19.25							
34g	top	44.97	9.05	17.12	80.28 ± 6.49	73	6.37^{yz}	6.00^{z}	6.16	5.65^{y}	5.96^{y}
	bottom	44.32	11.09	18.28							
Soybean oil											
40g	top	52.87	8.09	19.40	83.00 ± 4.60	77	6.80^{y}	6.72^{xy}	6.32	6.40^{x}	6.44^{xy}
	bottom	50.09	10.01	20.23							
34g	top	49.12	8.19	17.94	79.35 ± 8.61	73	5.99^{z}	6.11^{z}	6.36	6.00^{xy}	6.07^{y}
	bottom	47.02	10.58	19.11							
Vegetable shortening											
55g	top	45.83	10.54	17.83	81.67 ± 6.89	88	7.33^{x}	7.12^{x}	6.60	6.40^{x}	6.75^{x}
	bottom	47.84	11.24	19.69							
F Value:[d]							9.32***	5.49***	0.65	2.45*	2.76*

[a] per cookie
[b] Hedonic Ratings: 1 = dislike extremely; 9 = like extremely
[c] Within each sensory parameter, values with same letters are not significantly different ($p \geqq 0.05$)
[d] ***$p < 0.001$
* $0.01 < p < 0.05$

Table 4—Muffin characteristics

Muffin		Color L	Color a	Color b	Volume (ml)	Kcalories[a]	Sensory scores[b,c] Appearance	Flavor	Mouthfeel	Texture	Overall preference
n		2	2	2	3	1	75	75	75	75	75
Safflower oil											
36g	top	58.06	0.45	19.59	75 ± .90	168	5.80^{x}	5.38^{wxy}	5.80^{x}	5.90^{xy}	5.59^{xy}
	bottom	48.87	11.41	22.37							
27g	top	63.10	1.46	22.35	75 ± .43	162	6.13^{x}	5.18^{xyz}	5.55^{xy}	5.69^{xyz}	5.48^{xyz}
	bottom	59.93	10.47	22.85							
14g	top	64.34	1.50	22.53	75 ± .00	154	6.24^{x}	4.93^{yz}	5.08^{y}	5.20^{z}	5.05^{yz}
	bottom	46.58	11.88	20.93							
Soybean oil											
36g	top	58.83	5.22	23.29	75 ± .43	168	6.19^{x}	5.83^{w}	5.96^{x}	5.96^{x}	5.87^{x}
	bottom	50.55	10.87	21.99							
27g	top	51.33	8.07	21.78	75 ± .43	162	5.84^{x}	4.71^{z}	5.04^{y}	5.30^{yz}	4.94^{z}
	bottom	37.40	13.03	15.25							
14g	top	59.67	5.21	24.81	75 ± .00	154	6.15^{x}	5.73^{wx}	5.54^{xy}	5.78^{xyz}	5.80^{x}
	bottom	35.48	14.22	14.97							
Vegetable shortening											
53g	top	59.53	3.40	21.63	75 ± .43	178	4.92^{y}	5.94^{w}	5.69^{x}	5.51^{xyz}	5.61^{xy}
	bottom	50.82	10.11	22.03							
F Value:[d]							5.94***	5.64***	3.20**	2.23*	3.49**

[a] per muffin
[b] Hedonic Ratings: 1 = dislike extremely; 9 = like extremely
[c] Within each sensory paramter, values with same letters are not significantly different ($p \geqq 0.05$)
[d] *** $p < 0.001$
** $0.001 < p < 0.01$
* $0.01 < p < 0.05$

all three levels of safflower oil. Muffins made with vegetable shortening, 36 and 27g safflower oil and 36 and 14g soybean oil were preferred ($p < 0.05$) for mouthfeel. Additionally, there was no difference in preference of mouthfeel of muffins made with 14 and 27g of both oils. If one were to eliminate treatments which overlap in preference, results show that the 36g treatments of both oils (highest level of oils) and vegetable shortening were preferred the most, while lower oil level muffins were preferred less for mouthfeel. There was no difference in preference ($p > 0.05$) of texture of muffins made with 36 and 27g safflower, 36 and 14g soybean oil and vegetable shortening. Also, muffins made with 36 and 27g safflower oil and 27 and 14g soybean oil and vegetable shortening were judged to be similar in texture. The two lower levels of both oils (14 and 27g) and vegetable shortening were also judged similar. As with the other characteristics, there was overlap in results of the judges for overall preference.

There was variation in Gardner color scores for L, a and b values. The various levels and types of fat did not affect the muffin volume as all muffin treatments had a mean volume of

75 cc. Kcalorie content decreased directly with decrease in oil/ shortening content.

CONCLUSIONS

REDUCTION of the quantity of fat and the replacement of vegetable shortening by more highly polyunsaturated oils yielded a number of acceptable baked products. Additionally, the use of high polyunsaturated oils in baked products may have a nutritional advantage due to the high P/S ratio.

Yellow cakes made from commercial cake mix with various types and levels of oil/fat used in this study were judged equal in appearance, moistness, flavor and overall preference. All levels and types of oil/fat were found to be as acceptable for flavor, texture, color and overall preference in the pie crust formulation used in this study. However, all levels of types of oil/fat studied were not as acceptable in drop sugar cookies and muffins. All drop sugar cookies were considered similar in flavor, although cookies made with vegetable shortening were preferred for appearance, crispness and overall preference. Further investigation of the use of oils in cookies would perhaps yield more satisfactory results, especially if the oil cookies were not evaluated along with a standard cookie made of vegetable shortening. Muffin results were difficult to interpret because of overlap in sensory scores as well as varying color values. However, a number of oil treatments were as acceptable for a number of characteristics when compared with other muffin treatments. Muffins made with the oils were preferred for appearance. With the current recommendations for reduction of fat in the diet and increased use of polyunsaturated oils, the results of this study indicate that oil/fat levels in some products can be decreased without undesirable quality changes (or sensory changes).

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Chemical Composition and Nutritional Quality of Sugar Cookies Containing Full-Fat Sweet Lupine Flour (*L. albus* cv *Multolupa*)

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ABSTRACT

Full-fat sweet lupine flour (FFSL) (*Lupinus albus* cv Multolupa) containing 35.4% protein was used to enrich the cookies given to a group of school children. FFSL replaced 5, 10, 15, 20, and 25% of wheat flour in the formula. Farinological study, physical dough properties, chemical composition, and nutritional quality of cookies were studied. The protein content of the cookies increased by 8.9% up to 55.7% for the 5% and 25% FFSL, respectively. The highest PER (1.92) was found in cookies with 20% FFSL compared with 0.15 for the control. The results show a new area for application of FFSL in human diets.

INTRODUCTION

Cereals and their derivatives are staple foods in many countries and play a crucial role in feeding the world. Numerous studies have been conducted on the protein supplementation of wheat flour based foods. In particular, the incorporation of high lysine legume and oilseeds into bread and bakery products has been widely investigated and, in some cases adopted commercially (Hoover, 1975).

In Chile wheat and legumes are extensively consumed. About 35% of the protein and 40% of the calories, are derived from wheat. Legumes consumption (beans, lentils, chickpeas and peas) provides 4.2% of the calories and 8.3% of the protein (Salfate, 1980).

It has been demonstrated that the nutritive value of wheat flour protein is low but its amino acid profile can be improved through the addition of plant protein concentrates or lysine (Fleming and Sosulski, 1977). On the other hand legumes are deficient in S-containing amino acids but have an adequate lysine content. Thus, they might serve as a convenient complement for various bakery products.

Wheat based baked goods such as bread, cakes and cookies are popular foods and provide an excellent mean of improving the nutritional quality through incorporation of vegetable protein (Mc Watters, 1978).

Yáñez et al. (1983) have reported that sweet lupine flour (*L. albus* cv Multolupa) cultivated in Chile contains about 35% protein and a relatively high level of lysine. Studies in rats have shown its safety (Ballester et al., 1984). The prospect for sweet lupine as a high-protein human food seems promising (Lucisano and Pompei, 1981) and it may prove useful in the baking industry as a protein-rich supplement.

In Chile there is in operation the National Complementary Food Program (PNAC) that distributes free foods to pre-school and school children of low socioeconomic level (González and Sánchez, 1982; Olivares et al., 1984). For the last three years a daily average of 750,000 breakfasts and 250,000 lunches were served for 180 days a year. Cookies are one of the foods they received in a quantity of 30-40 g/day during 180 days per year. Therefore it appears that cookies supplemented with lupine might be of value in improving its nutritional quality.

This study was designed to evaluate the effect of the incorporation of various concentrations of full-fat sweet lupine flour on baking, chemical and nutritional characteristics of cookies.

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Table 1—Chemical analysis of wheat flour (WF) and lupine flours (FFS)

	WF g/100g	FFSL g/100g
Moisture	11.8	6.8
Ash	0.6	3.6
Protein	10.1 (N × 5.7)	37.4 (N × 6.25)
Ether extract	1.5	11.2
Crude fiber	0.3	1.9
N-free extract[a]	75.7	39.1

[a] By difference

MATERIALS & METHODS

Materials

Sweet lupine flour (*L. albus* cv Multolupa) (FFSL) containing 0.02% total alkaloids was a full-fat commercial sample obtained at the Campex Baer located in Gorbea (Chile). Wheat flour (WF) was purchased at the local market.

Cookies

The basic formula employed for the cookie preparation was: all purpose wheat flour (76.7 g); vegetable shortening (10.5g); malt syrup (1.5 g); sugar (18.0 g); salt (NaCl) (300 mg); sodium bicarbonate ($NaHCO_3$) (500 mg); calcium phosphate ($CaHPO_4$) (200 mg); vanillin (100 mg); water (8.7 mL).

Preparation of cookies

Cookies containing 0, 5, 10, 15, 20, and 25% FFSL were prepared in a local plant participating in the National Complementary Food Program. These concentrations replaced the wheat flour in the basic formula (76.9 WF). Wheat flour, FFSL, $NaHCO_3$, $CaHPO_4$ and vanillin were mixed in a NRC planetary type mixer. Melted hydrogenated fat and sugar solution (2:1) and a part of the water were added into the mixing bowl. This was then made up into a smooth paste with the gradual addition of the remaining water. The finished dough was shaped in a cookie molder and baked on sheets for 10 min at 270°C in an electric oven. Baked cookies were cooled to room temperature (20°C) and immediately evaluated for physical parameters.

Analytical procedures

Proximate analysis. The materials and diets used in this study were analyzed for moisture, ether extract, total ash, nitrogen and crude fiber according to standard AOAC methods (AOAC, 1980).

Protein was calculated by using the factors N × 5.7 for wheat flour and N × 6.25 for FFSL. The latter factor is generally used for legumes.

Calories were calculated applying the factors, 4, 9, 4 of Atwater for protein, lipid and carbohydrates, respectively.

Farinological study. Mixing properties of the lupine-wheat flour blends were studied in a Brabender farinograph. Dough water absorption and dough mixing time were also measured. The results were expressed as Valorimeter Value (W). This is an empirical single-figure quality score based on dough development and tolerance mixing.

Physical parameters. Weight (g), diameter (cm) and height (cm) were measured on an average of ten cookies for each FFSL level and spread ratio was calculated as the diameter to height ratio.

Biological study

For the feeding test in rats, dried and ground samples were incorporated into experimental diets with calculated amounts of vitamins

Table 2—Proximate analysis of cookies supplemented with varying levels of sweet lupine flour (FFSL)

	FFSL (%)					
	0	5	10	15	20	25
Moisture (g/100 g)	2.2	3.2	3.3	3.3	3.1	4.5
Ash	1.1	1.2	1.3	1.4	1.5	1.6
Protein (N×5.7)	7.9	8.6	9.8	10.6	11.6	12.3
Ether extract	12.3	11.9	12.1	13.2	13.5	13.4
Crude fiber	0.4	0.6	0.6	0.7	0.8	1.0
Nitrogen-fre extract[a]	76.1	74.5	72.9	70.8	69.5	67.2
Calories (Kcals/100 g)	447	440	440	444	445	439

[a] By difference

Table 3—Farinograph properties of blends of wheat flour and lupine flour (FFSL)

	FFSL (%)					
	0	5	10	15	20	25
Water absorption (%)	60.6	64.4	64.9	67.6	71.0	71.8
Farinograph values						
Developing time (min)	4.5	4.0	3.0	4.5	3.5	4.0
Weakening (BU)	100	100	110	120	130	120
Valorimeter value (W)	51	47	42	44	44	46

Table 4—Physical characteristics of sugar cookies prepared from blends of wheat flour and lupine (FFSL)[a]

Cookie formula	Weight (g)	Diameter (mm)	Height (mm)	Spread ratio[d]
Control	6.79[a]	45.9	9.2	4.97[a]
FFSL (% wheat flour replaced)				
5	6.61[ab]	46.7	9.4	4.99[a]
10	6.60[ab]	45.7	8.8	5.16[b]
15	6.63[ab]	45.9	9.1	5.06[ab]
20	6.49[bc]	45.3	8.5	5.32[c]
25	6.47[bc]	45.5	8.6	5.28[c]

[a-c] Mean of five cookies selected at random.
Means in column with the same letter are not significantly different (p>0.001)
[d] Diameter/height.

Table 5—Protein efficiency ratio (PER) of cookies with different levels of FFSL

Cookies	Dietary protein (N×5.7) (g/100g)	Weight increase (g)	PER
Control	6.5	2.4 ± 3.3[a]	0.15 ± 0.25[a]
FFSL (%)			
5	6.7	14.0[b] ± 4.0	1.08[b] ± 0.20
10	7.4	20.0[b] ± 3.8	1.34[c] ± 0.24
15	8.5	31.7[c] ± 4.8	1.47[c] ± 0.17
20	9.3	52.6[d] ± 4.5	1.92[d] ± 0.09
25	9.7	49.0[d] ± 3.5	1.81 ± 0.13

[a] Mean ± S.D. mean in same column with the same letter are not significantly different (p>0.01).

and mineral mixtures according to Chapman et al. (1959) with the modification of Elías for cereal foods (Elías et al., 1974). Three-week-old weanling rats (Wistar) were fed experimental diets for 28 days. The rats, whose initial weight averaged 52.2g were randomly divided into groups of eight per treatment. Each rat was housed in a screen-bottom cage in an environmentally controlled laboratory and given a test diet and water ad libitum. Fresh water was supplied every day; feed cups were checked daily and refilled as needed. Weights and feed consumed were recorded weekly for each rat and PERs were calculated. Feed wastage was subtracted from consumption data.

Statistical analysis

The data were subjected to analysis of variance according to Snedecor and Cochran (1967) and Duncan's multiple range test (1955).

RESULTS & DISCUSSION

Proximate analysis

The proximate chemical data of materials (Table 1) showed no differences from our previously reported values (Ballester et al., 1980). The incorporation of FFSL modified the chemical composition of cookies especially in moisture and protein (Table 2). Moisture increased from 2.2% for the control to 4.5% for the 25% level and the protein from 8.6% for the 5% FFSL to 12.3% for the 25% level. Lupine flour did not modify the energy value of cookies (Table 2).

Farinological study

Table 3 lists the results of the farinological study on flour mixtures containing different levels of FFSL. The amount of water (absorption) required to center the farinogram curve on the 500 BU (Brabender Units) line increased steadily with every increment of FFSL from 60.6% for 0% FFSL to 71.8% for the 25% level, probably due to the hygroscopic properties of protein that are in higher proportion in FFSL and/or to the extra protein.

The dough developing time decreased with the addition of lupine from 4.5 min for wheat flour to 3 min for the 10% FFSL. From the industrial point of view this is important especially for making cookies that require a dough with short developing time. Higher levels of FFSL increased developing time to 4 min for the 25% FFSL. These results may imply that the physical mechanical properties of the dough such as viscosity, elasticity, plasticity and consistency were slightly modified by the incorporation of lupine flour. Weakening of the dough is inversely proportional to the gluten content of the flour. The increased values found for this parameter in FFSL-WF could be attributed to the higher lupine content. This flour like that of other legumes, lacks gluten.

A good quality bakery flour has a valorimeter value (W) of 40, obtainable from wheat flours containing approximately 8–9% (N×5.7) protein at the most. In the present study we used a moderately hard wheat flour containing 10.1% protein which could explain the W value of 51 for this product. Mixing wheat flour with FFSL reduced the gluten content in WF thus lowering the W value. The blend containing 10% FFSL reached the lowest value which came closer to the recommended level of 40.

Physical parameters

Table 4 shows the results of physical data of sugar prepared from wheat and FFSL. It was observed that the weight was significantly ($p<0.001$) less for FFSL cookies. Spread ratio, as an indicator of cookie density, significantly ($p<0.001$) increased for cookies with 10, 20, and 25% FFSL compared with the control.

—*Continued on page 658*

Sensory and Nutritional Evaluation of Wheat Bread Supplemented with Single Cell Protein from Torula Yeast (*Candida utilis*)

JOHN CHING-MING LIN, MARIAN F. CHASTAIN, and D.R. STRENGTH

ABSTRACT

Experimental breads were made with 2, 4, 6, 8, 10, and 12% torula yeast flour (TYF). Taste panels found that bread fortified with 8% TYF was acceptable. Eight percent TYF raised protein content from 12.42% for all wheat flour bread to 14.22% for supplemented bread. Amino acid analyses indicated a marked increase in amino acid content especially lysine. The protein efficiency ratio (PER) for rats on all-wheat-flour bread was 1.31; replacing 8% of the wheat flour with TYF raised PER of supplemented bread to 2.28. Total carcass nitrogen retained by animals fed a diet containing supplemented bread was higher than that retained by rats whose protein was obtained from unsupplemented bread.

INTRODUCTION

PROTEIN MALNUTRITION is a serious problem facing people whose diets consist mainly of cereals or other starchy products. To help alleviate the problem, many investigators have studied new and unusual sources of protein. Since bread is consumed by people of practically all cultures and in all countries, it would be a practical vehicle for nutritional enrichment of diets. Protein-enrichment of bread has been reviewed by Pomeranz (1970). The combination of wheat flour with another protein source offers a possible economical improvement of protein quality of wheat bread as well as greater total protein content (Finney et al., 1950; Chastain et al., 1975; Carlson et al., 1981; Elgedaily et al., 1982; Kvitka and Chen, 1982; Repetsky and Klein, 1982; Ballester et al., 1984).

The use of single cell protein from yeast as a supplement has been investigated in numerous studies (Klapka et al., 1958; Yanez et al., 1972; Young and Scrimshaw, 1975; Eroshin et al., 1977; Lindblom, 1977; Kiovurinta, 1978). The species most commonly used in the production of yeast protein are *Candida utilis* (Torula yeast), *Saccharomyces cerevisiae* and *Saccaromyces fragilis* (Frazier, 1967). Food yeast is high in protein, minerals and most of the B complex vitamins. The protein content of *Candida utilis* grown on ethanol is approximately 50% on a dry weight basis (Anonymous, 1977). This concentration compares favorably with fish meal or soy meal (MacLaren, 1975).

The amino acid composition of torula yeast protein compares well with the FAO pattern except for its low content of methionine (FAO/WHO, 1973). Yeast protein is generally considered to be inadequate in sulfur-containing amino acids but a good source of lysine (Lipinsky and Litchfield, 1974). Wheat flour and other cereal proteins, by comparison, are adequate in methionine and cystine but low in lysine. Since the amino acid composition of yeast protein and wheat flour are highly complementary, there is good potential for the use of yeast protein in cereal product supplementation (Mateles and Tannebaum, 1968). Studies by Yanez et al. (1972) have shown the Protein Efficiency Ratio (PER) in rats of bread with 10% *Candida utilis* yeast cultivated in beet molasses to be 1.74 as compared with 0.84 for unsupplemented bread.

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Table 1—Basic recipe for experimental breads

Ingredients	Amounts
Bread flour	370.0 g
Sugar	19.5 g
Salt	7.5 g
Hydrogenated vegetable shortening	12.0 g
Baker's yeast (active, dry)	3.5 g
Milk[a]	118.0 mL
Water	118.0 mL

[a] Reconstituted nonfat dry milk.

Preliminary studies (Lin, 1982) done prior to the research reported here showed that xylose was a good substrate for the growth of torula yeast. Xylose is a component of hemicellulose and the latter is readily available in large amounts as a by-product of the wood products industry. The torula yeast used in this study was a commercial product grown on ethanol. The objectives of this research were: (1) development of a bread fortified with torula yeast protein with acceptable sensory characteristics; (2) assessment of the nutritional quality of the high protein bread as measured by PER and amino acid analysis.

MATERIALS & METHODS

Bread preparation

Breads containing seven levels of Torula yeast flour (Torutein - 10, Amoco Foods Company, Chicago, IL) were prepared by replacing 2, 4, 6, 8, 10, and 12% of the wheat flour (WF) with TYF in a standard yeast bread formula (Table 1). Mixing was done by a conventional straight dough method.

After initial experiments showed the highest acceptable level of TYF to be 8%, attempts to mask torula yeast flavor by various substitutions were undertaken. Whole wheat flour or oat flour was substituted for 25% of the bread flour, and honey or molasses was substituted for the granulated sugar (using the same weight of sweetening on the dry weight basis).

Breads were baked in a household type electric oven. To obtain the same degree of browning in all bread, it was necessary to adjust the oven temperature and baking time for higher TYF levels. Bread with TYF resulted in a darker crust than the control; therefore, breads fortified with TYF were baked at 350°F (177°C) rather than 400°F (205°C). After baking, loaves were cooled, packaged in plastic bags and stored overnight at approximately 20°C, or in some cases in the freezer at − 18°C for a longer time.

Volume measurement

Loaf volume of the baked breads was determined by cutting each loaf in half crosswise and measuring the height in centimeters at the outer edges, at the center, and at points one-half the distance from the center to the edges. The mean of these five measurements was then recorded as Index of Volume (Funk et al., 1969).

Sensory evaluation

So that judges would not be influenced by the variations in crust color, crusts were removed from the bread slices prior to evaluation. An eight member panel consisting of faculty and staff of the Nutrition and Foods Department was used for the first evaluation of acceptability of the experimental bread. Bread with 8% TYF was judged for color, flavor and moistness. Judges were asked to check the descriptive term which represented their rating of the product. For tabulation and statistical treatment, numerical values (1–5) were assigned to the

Table 2—Composition of test diets

	Casein g/kg diet	0% TYF bread g/kg diet	8% TYF bread g/kg diet
Protein	115	829	721
Cornstarch	714	—	108
Sugar	23	23	23
Vitamin mix[a]	20	20	20
Salt mix[b]	50	50	50
Lard	50	50	50
Ascorbic acid	1.0	1.0	1.0
Agar	10	—	—
Penicillin G	0.1	0.1	0.1
Alphacel	20	20	20
Water	24	24	24

[a] Contained the following: Vitamin A (retinol palmitate) - 10,000 I.U./kg, Vitamin D (ergocalciferol) - 1,600 I.U./kg, Vitamin E (α-tocopherol succinate) - 55 I.U./kg, Vitamin K_1 - 50 mcg/kg, Biotin - 0.5 mg/kg, Choline - 3000 mg/kg, Folic Acid - 2 mg/kg, Inositol - 200 mg/kg, Niacin - 100 mg/kg, Ca Pantothenate - 50 mg/kg, Riboflavin - 10 mg/kg, Thiamin - 20 mg/kg, Vitamin B_6 - 24 mg/kg, Vitamin B_{12} - 50 mcg/kg.

[b] Contained the following major minerals: Calcium - 7.33 g/kg, Chloride - 1.11 g/kg, Magnesium - 0.81 g/kg, Phosphorus - 5.68 g/kg, Potassium - 4.68 g/kg, Sodium - 1.91 g/kg, Sulfur - 1.06 g/kg; and the following trace minerals: Copper - 16 mg/kg, Fluoride - 2 mg/kg, Iodide - 20 mg/kg, Iron - 200 mg/kg, Manganese - 80 mg/kg, Selenium - 0.2 mg/kg, Zinc - 56.0 mg/kg.

various terms: 1 = very poor color, very poor flavor, dry; 5 = very good color, very good flavor, gummy.

A second set of sensory evaluations of bread with 8% TYF was done using a twelve number panel consisting of students majoring in Nutrition and Foods who had had some experience in taste panel work. Food action (FACT) rating score sheets were used (Schutz, 1965). FACT requires the individual to indicate his reaction to the food in one of the following terms: I would eat this every opportunity I had; I would eat this very often; I would frequently eat this; I like this and would eat it now and then; I would eat this if available but would not go out of my way; I don't like it but would eat it on an occasion; I would hardly ever eat this; I would eat this only if there were no other food choices; I would eat this if I were forced to. The first time the bread was evaluated by the FACT method, it was served plain and panelists were told nothing about the product. One week later, the same panelists evaluated identical bread samples but were provided with butter and grape jelly to use on the bread as they desired. Ratings on the bread were then carried out. Since the butter and jelly would provide kilo calories and this group of panelists was extremely calorie conscious they were instructed to use butter and jelly as desired without consideration of extra calories. For the third evaluation the panelists were told that the bread was high-protein bread. They were asked how would they feel about the product if that bread were the main source of protein in their diets. For tabulation and statistical treatment, numbers of 1–9 were assigned to the various statements: 1 = I would eat this if I were forced to; 9 = I would eat this every opportunity I had.

The 8% TYF bread with whole wheat flour, honey or molasses was judged by a panel composed of eight Nutrition and Foods graduate students. Breads were evaluated on a graphic scale (0–10) with 0 = coarse grain, undesirable flavor, undesirable mouthfeel; 10 = fine grain, desirable flavor, desirable mouthfeel.

Chemical analysis

Breads containing TYF at levels of 0% and 8% were broken by hand into pieces approximately 1 in. (2.5 cm) in diameter and were dried in a hot air oven at 56°C for 24–48 hr to stabilize the moisture content. The dried bread samples were ground in a Wiley Mill and blended under ambient temperature and moisture conditions. Moisture content was determined after heating the ground blended samples at 100°C for 24 hr. Nitrogen determinations were done on the dried samples by the macro-Kjeldahl method (AOAC, 1980).

Nutritional evaluation

Dried ground portions of 0% and 8% TYF bread were incorporated into rat diets which were formulated to contain 10% protein (Strength, 1970). A control diet was prepared using casein as the protein source. Diets were mixed in quantities of 1 kg and stored at 4°C until used. The diet composition is shown in Table 2.

Twenty-four 3-week old weanling male albino rats of the Charles River CD strain were assigned at random to four different groups with six rats per group. They were housed individually in screen-bottom cages in an environmentally controlled room, with a temperature of 22–24°C, humidity of 50%, and a 12 hr light/12 hr darkness cycle. At the end of a 3-day stabilization period on commercial ration, one group was sacrificed. Carcasses were wrapped in aluminum foil and frozen for later total nitrogen determinations. The remaining three groups were fed the experimental diets and were similarly sacrificed at the end of the experimental period.

Table 3—Effect of Torula yeast (TYF), whole wheat (WWF), and oat (OF) flours on loaf volume

Flour	Index of Volume[a] (cm)
100% White	10.36[b] + 0.87
98% White + 2% TYF	10.01[b] ± 0.61
96% White + 4% TYF	9.68[b] ± 0.56
94% White + 6% TYF	8.48[b] ± 0.39
92% White + 8% TYF	7.21[b] ± 0.35
90% White + 10% TYF	6.29[b] ± 0.33
88% White + 12% TYF	5.26[b] ± 0.45
69% White + 23% WWF + 8% TYF	7.10[c] ± 0.83
69% White + 23% OF + 8% TYF	4.60[c] ± 1.17

[a] Mean of 5 height measurements on cut surface (Funk et al., 1969).

[b] Each value is the mean ± standard deviation of measurements on 5 loaves.

[c] Each value is the mean ± standard deviation of measurements on 3 loaves.

The rats were fed immediately after being placed in individual cages and daily thereafter. Glass distilled water was supplied *ad libitum*. Feed consumption and body weights were recorded daily for three weeks. Animals were weighed at approximately the same time daily, and at that time the feed jars were removed, weighed and replenished. Feed wastage was subtracted from consumption data. Animals were killed by ether anesthesia and the carcasses were frozen at the end of the 21-day test period.

The Protein Efficiency Ratio (PER) was calculated as weight gain in grams for each gram of protein (N × 6.25) consumed. The individual PER values were determined for the animals after 3 weeks on the experiment, and group means were calculated from individual PER values.

For total carcass nitrogen determination, frozen carcasses of animals in each test group were allowed to thaw at room temperature. Fissures were made in the head and abdomen to facilitate drying, which was done at 100°C for 48 hr after which the carcasses were ground. Moisture determinations were done on pooled samples from each group. Nitrogen content was determined on aliquots of the pooled samples by the macro-Kjeldahl method (AOAC, 1980).

Amino acid determination

Amino acid estimations of the bread samples were made by means of a Beckman 121 M Automatic Amino Acid Analyzer (Beckman Instruments, Palo Alto, CA). Hydrolysis was carried out by the procedure of Robbins et al., (1971). Analyses were replicated six times.

Statistical analysis

Data were analyzed using analysis of variance to determine if significant differences ($p \leq 0.05$) existed among means (Snedecor and Cochran, 1980). To locate the differences among means, Duncan's (1955) New Multiple Range Test was applied to the data.

RESULTS & DISCUSSION

Volume and sensory characteristics

The index of volume measurement confirmed the visual observation that TYF decreased loaf volume (Table 3 and Fig. 1). With increasing amounts of TYF, the height of the ends of loaves decreased sharply. In some trials an unsymmetrical shape occurred in breads containing 10 to 12% TYF. Whole wheat flour or oat flour substituted for part of the bread flour in 8% TYF bread also decreased loaf volume (Table 3). Bread with oat flour substitution was heavy with a very compact grain; and sensory qualities were not studied.

Crust color of all bread supplemented with TYF was darker than that of the control bread (0% TYF). Interior color of TYF bread was golden yellow which was considered desirable by most judges. The preference for the yellowish crumb color of TYF bread might be a pyschological effect because of perceptions that off-white breads are often more nutritious. Yellow color was due partially to the color of TYF itself and also to

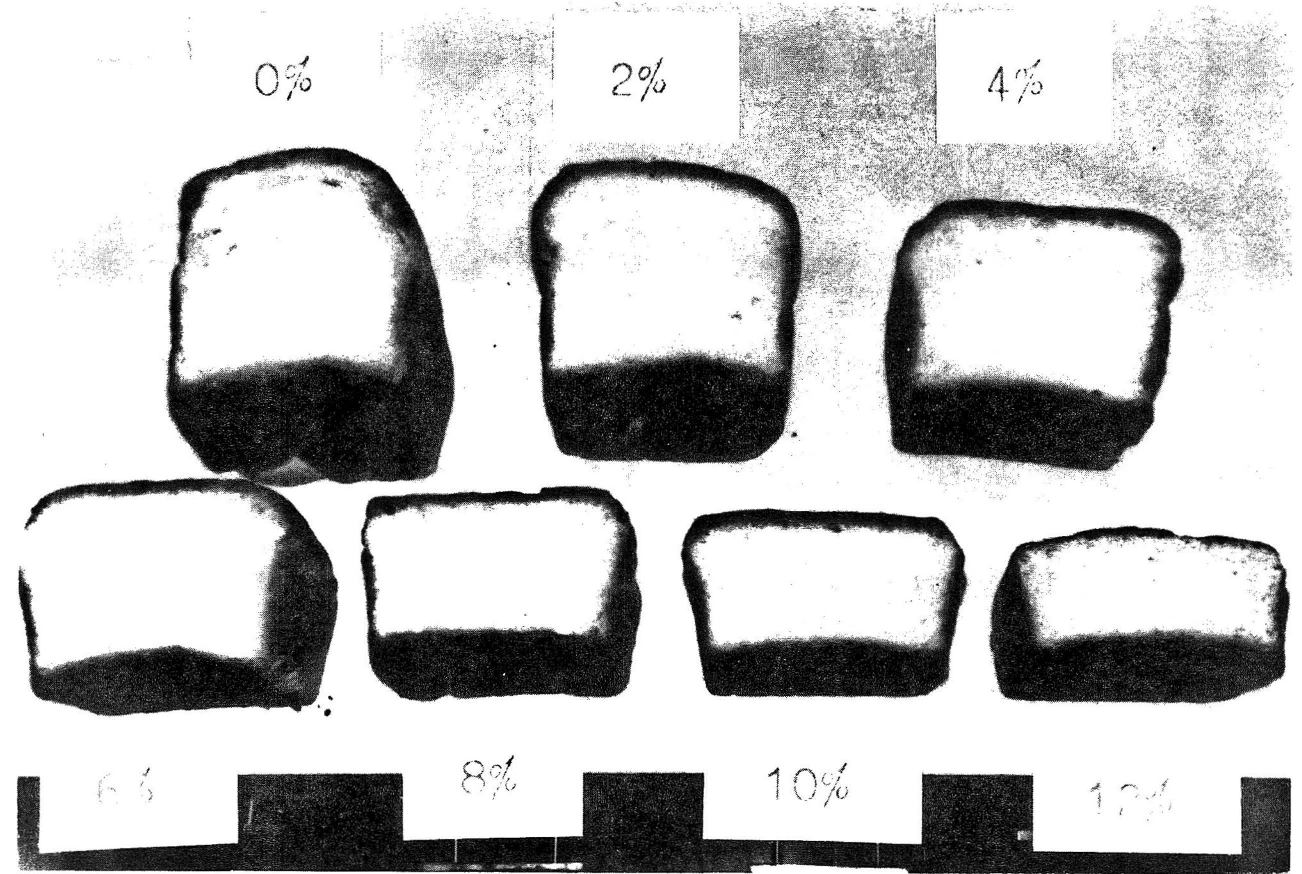

Fig. 1—Effect of various levels of torula yeast flour on bread volume.

Table 4—Initial sensory evaluation of 8% TYF bread[a]

	Score	Descriptive rating
Color[b]	4.2 ± 1.5	good
Flavor[b]	3.0 ± 0.5	fair
Moistness[c]	3.0 ± 0.4	pleasingly moist

[a] Each value is the mean ± standard deviation of eight judgements.
[b] Scale 1 - 5 (1 = very poor, 2 = poor, 3 = fair, 4 = good, 5 = very good).
[c] Scale 1 - 5 (1 = dry, 3 = pleasingly moist, 5 = gummy).

Table 5—Results of FACT[a] rating test on 8% TYF bread[b]

	Score	Descriptive Rating
Bread served plain, unidentified	6.4^{x} ± 1.5	I like this and would eat it now and then.
Bread served with butter and jelly	8.2^{y} ± 0.6	I would eat this very often
Bread served plain, identified	7.9^{z} ± 1.3	I would frequently eat this.

[a] Schutz, 1965.
[b] Each value is the mean ± standard deviation of twelve judgments.
x,y,z Means in the same column with different superscripts are different ($p \leq 0.05$).

the heat-accelerated Maillard reaction. The higher protein content of the TYF bread resulted in greater browning.

The grain of breads containing up to 8% TYF was typical of standard quality wheat bread. However, as levels of TYF were increased above 8%, air cells became larger and the grain became coarser; breads were heavier with a compact consistency at loaf bottom; and loaves broke apart readily. Breads containing 10 and 12% levels of TYF were unacceptable both from the standpoint of texture and flavor. The dough failed to develop elastic properties when kneaded and did not rise properly in proofing and in baking. This was probably due to reduced gluten development resulting in failure to retain gas, which in turn caused the heavy loaf with poor volume.

Results of initial sensory evaluation of 8% TYF bread are shown in Table 4. The flavor of TYF in the bread was readily detectable even at the 8% level. This flavor was described by various individuals as being "yeasty," "salty" or "bitter" with some tasters thinking the characteristic flavor was more of an aftertaste rather than detectable initially. Eight percent TYF bread had a satisfactory color, desirable degree of moistness, and a fair flavor. Based on these results further sensory studies with 8% TYF bread were done.

The results of the FACT rating test are shown in Table 5. When judges were served the plain 8% TYF bread, the bread received a 6.4 rating corresponding to "I like this and would eat it now and then." When panelists had butter and jelly available and thus were able to mask the torula yeast flavor, they indicated: "I would eat this very often" which in numerical terms was an 8.2 rating. The difference of the means was statistically significant ($p \leq 0.05$).

The acceptability of the TYF bread was possibly influenced by mental comparison with all wheat bread. When test bread was identified as high-protein bread that could be the main source of protein in the diet for some people, the scores were significantly improved ($p \leq 0.05$). The scores were higher than for unidentified bread but not as high as when butter and jelly were available to mask the flavor. Although persons in developing countries might not have butter and jelly, they would possibly have some flavorful low nutritive value products which would mask the flavor of high-protein bread.

Table 6 shows the results of attempts to mask the TYF flavor by using whole wheat flour, honey and molasses. In this part of the study, panelists received bread samples which had been held in frozen storage for several weeks. The average results of panel scores showed that judges significantly preferred the flavor of the breads containing whole wheat, honey, or molasses, compared to the basic TYF bread. Pungency of flavor

Table 6—Acceptability of TYF bread with whole wheat flour, honey and molasses[a]

Bread	Flavor[b]	Mouthfeel[b]	Grain[c]
TYF, basic	$5.0^w \pm 0.4^a$	$5.3^w \pm 1.6$	$5.0^w \pm 0.2$
TYF with whole wheat flour	$6.0^x \pm 0.6$	$6.2^w \pm 3.0$	$4.5^w \pm 0.4$
TYF with honey	$7.3^y \pm 1.0$	$5.6^w \pm 3.5$	$5.6^w \pm 0.8$
TYF with molasses	$7.2^z \pm 0.9$	$5.4^w \pm 3.2$	$5.2^w \pm 0.7$

[a] Each value is the mean ± standard deviation of eight judgements.
[b] Scale 0 - 10 (0 = undesirable, 10 = desirable).
[c] Scale 0 - 10 (0 = coarse, 10 = fine).
[w,x,y,z] Means in the same column with different superscripts are different ($p \leq 0.05$).

Table 7—Protein content of test breads

TYF in Bread	Protein					
	Bread Ingredients				Bread	
%	TYF[a] %	WF[b] %	Milk %	BY[c] %	Calculated %	Kjeldahl[d] %
0	0	9.93	2.19	0.30	12.42	13.44 ± 0.20
8	2.60	9.13	2.19	0.30	14.22	18.70 ± 0.14

[a] TYF = Torula Yeast Flour.
[b] WF = Wheat Flour.
[c] BY = Baker's Yeast.
[d] Each value is the mean ± standard deviation of 3 determinations.

is associated with molasses, thus molasses gives a special kind of flavor to the TYF bread which the panelists found to be desirable.

Mouthfeel of whole wheat bread with TYF was rated highest and grain of that bread was rated lowest but none of the mouthfeel and grain differences were significant.

Protein levels

The protein content of breads as calculated from published values for the bread ingredients (Church and Church, 1975), along with the values as determined by Kjeldahl (N × 6.25) are shown in Table 7. TYF bread contained a higher percentage of crude protein than did 100% WF bread due to the higher protein content of TYF as compared to WF. Milk and baker's yeast also contributed small amounts of protein to the breads.

The crude protein contents derived from Kjeldahl determination (N × 6.25) of WF bread and TYF bread were 13.44% and 18.70%, respectively. However, the protein contents calculated from published values (Church and Church, 1975) 12.42% for WF bread and 14.22% for TYF bread, were lower. The difference in the values determined by the two methods was greater for the TYF bread than for the WF bread. Yang et al. (1977) pointed out that protein content of microbial cells calculated from Kjeldahl nitrogen was an overestimate of the protein, since nitrogen from nucleic acid and hexosamine were included. Therefore, the factor of 6.25 used to convert the Kjeldahl nitrogen to the crude protein values may not reflect the true nature of microbial protein.

Nutritional quality

Means for weight gain, protein consumption and PER values are shown in Table 8. As expected, the response of rats fed the casein diets was greater than that of rats on either of the bread diets. When bread with TYF was fed as the sole source of nitrogen in the diet, at the 10% protein level, the 21-day weight gain (71.16 g) was significantly ($p \leq 0.05$) greater than that (30.16 g) of the animals receiving bread without TYF. However, the weight gain of the animals fed the diets containing bread as a protein source was lower than that of those on casein diets. There was significantly ($p \leq 0.05$) greater protein consumption on the 8% TYF bread diet than on the bread diet without TYF. However, the casein diet produced the highest level of protein consumption among the three experimental diets.

The casein PER value of 2.95 compares favorably with a PER of 3.08 reported by Shehata and Fryer (1970). The PER values of WF bread and TYF bread were 1.31 and 2.28, respectively. The addition of TYF increased significantly ($p \leq 0.05$) the nutritional quality of the bread protein. On the basis of PER in young rats, the nutritional value of bread with TYF was superior to that of bread without TYF.

Table 9 shows carcass nitrogen (N) data for the pooled rat carcasses from each dietary treatment. Carcasses of rats on all experimental diets contained more N than rats killed at the beginning of the experimental period. Total body N content of animals fed WF bread and TFY bread were 15.08g and 22.26g, respectively. While neither of the bread diets resulted in as much N accumulation as the casein diet, the 8% TYF bread produced significantly greater body N retention than did bread without TYF. Subtraction of the carcass N of the base group of animals from carcass N for each of the experimental groups shows the amount of N retained by each group. Comparison of N retention and N consumption data gives the relative N efficiency for each diet. Although the 8% TYF diet was not as efficient as the casein diet, it showed a marked improvement over the WF diet.

Of possible concern is the nucleic acid (NA) content of TYF which has been reported to contain 8% ribonucleic acid (RNA) (Gierhart and Potter, 1978). Using TYF at a level of 8% in the bread resulted in 2.4 g RNA per loaf or 0.2g per slice (12 slices per loaf). The Protein Advisory Group (1970) suggested that 2g NA per day added to the normal mixed diet would be an upper safe limit for healthy young adults. A person would have to eat more than 10 slices of bread daily to exceed the suggested limit.

Amino acid analyses

In the WF bread, 80.0% of the total protein was from wheat flour; in the TYF bread, 64.2% of the total protein was from wheat flour. As might be expected, a change in amino acid proportions resulted. Analysis data for seven essential amino acids are shown in Table 10. Procedures used in this study did not include a determination of tryptophan. The levels of all amino acids were increased by the inclusion of the torula yeast product. This is particularly important in the case of lysine, the most limiting amino acid in wheat protein.

CONCLUSIONS

ALTHOUGH TYF was found to depress loaf volume, bread made with 8% TYF had acceptable volume, flavor and moistness. Panelists gave higher ratings to bread supplemented with

Table 8—Performance of rats fed torula yeast bread diets for a 3-wk period[a]

Diet	Weight gain (g)	Protein consumption (g)	PER	Adjusted[b] PER
0% TYF bread	$30.16^x \pm 6.79$	$22.98^x \pm 2.30$	$1.31^x \pm 0.21$	1.11
8% TYF bread	$71.16^y \pm 11.21$	$31.17^y \pm 2.73$	$2.28^y \pm 0.17$	1.90
Casein	$122.83^z \pm 5.71$	$41.61^z \pm 1.68$	$2.95^z \pm 0.10$	2.50

[a] Each value is mean ± standard deviation of 6 determinations.
[b] Adjusted to standard AOAC value for casein.
[x,y,z] Means in the same column with different superscripts are different ($p \leq 0.05$).

Table 9—Effect of diet on rat carcass nitrogen

Diet	Total group[a] dry body wt g	Total[a] carcass N per group g	Net N retained per group g	Total N consumed per group g	Relative[c] N efficiency %	Mean N[a] carcass wt per 100g g
Base[b]	101	9.50[w]	---	---	---	9.41 ± 0.43
WF	208	15.08[x]	5.58	22.06	25.30	7.25 ± 0.63
8% TYF	305	22.26[y]	12.76	29.92	42.64	7.30 ± 0.61
Casein	421	32.50[z]	23.00	39.95	57.57	7.72 ± 0.40

[a] Each value is the mean ± standard deviation of 6 determinations.

[b] Base - Not fed experimental diet; animals sacrificed and analyses done at beginning of experimental period. Animals fed test diets were analyzed at end of 3 weeks.

[c] Relative nitrogen (N) efficiency = N retained ÷ N consumed × 100.

[w,x,y,z] Means in the same column with different superscripts are different ($p \leq 0.05$).

Table 10—Essential amino acids in test breads

	0% TYF Bread		8% TYF Bread	
	mg[a]	g[b]	mg[a]	g[b]
Isoleucine	284 ± 16.97	2.28	606 ± 38.18	4.26
Leucine	524 ± 27.28	4.22	787 ± 31.83	5.53
Lysine	100 ± 17.67	0.81	316 ± 26.16	2.22
Methionine	34 ± 10.60	0.27	72 ± 16.26	0.51
Phenylalanine	334 ± 38.18	2.69	680 ± 35.35	4.78
Threonine	209 ± 44.55	1.68	570 ± 27.30	4.01
Valine	310 ± 35.47	2.49	578 ± 31.12	4.06

[a] mg amino acid per 100 g bread. Each value is the mean ± standard deviation of 3 determinations.

[b] g amino acid per 100 g protein.

8% TYF when they had butter and jelly to mask the flavor. When bread was identified as a high-protein product, panelists rated it higher than they did when they did not know the purpose of the study.

Addition of 8% TYF to bread increased the protein content from 12.42% for all wheat flour bread to 14.22% for supplemented bread. Amino acid analyses indicated a marked increase in amino acid content, especially lysine, the limiting amino acid in wheat flour. The PER of all wheat flour bread was 1.31; replacing 8% of the wheat flour with TYF raised the PER of the bread to 2.28. The total carcass nitrogen retained by rats on the experimental bread diet was higher than that of rats on bread without supplementation.

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Survival of *Campylobacter jejuni* in Selected Gaseous Environments

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ABSTRACT

Vacuum-packaged and oxygen-permeable, polyvinyl chloride film wrapped broiler chickens were compared with one another to determine influence on the survival of *Campylobacter jejuni*. No significant (P<0.01) difference in the survival of *C. jejuni* was observed, irrespective of the packaging system assessed by either surface rinse or drip sampling. Significantly (P<0.01) greater numbers of the organism were recovered by surface rinse sampling in both packaging systems when compared with the numbers obtained from drip sampling. Recovery of indigenous *Campylobacter* from red meat carcass samples was greater in the vacuum packaged samples (19/63) than in the oxygen permeable wrapped samples (7/63) after the samples were held at refrigeration temperatures for 1 wk. Significantly (P<0.01) greater survival of *C. jejuni* in ground beef was demonstrated in a 100% nitrogen atmosphere when compared with a vacuum, a 80% carbon dioxide and 20% nitrogen atmosphere or a 5% oxygen, 10% carbon dioxide and 85% nitrogen atmosphere. These results indicate that the organism survives at variable rates in different atmospheres, but these differences were relatively small and unlikely to impact on the public health.

INTRODUCTION

CAMPYLOBACTER JEJUNI is established as an enteropathogen responsible for endemic and epidemic enteritis in humans. The epidemiologic evidence of *Campylobacter* induced disease indicates that both foods of animal origin and improperly treated waters serve as important vehicles in the transmission of the infectious agent to man (Brouwer et al., 1979; Oosterom, 1981; Tiehan and Vogt, 1978). The organism is present as a commensal in the intestinal tract of domestic livestock animals (Hänninen and Raevuori, 1981; Smibert, 1965) and typically does not manifest any pathological signs in these animals.

During slaughter and meat processing, intestinal contents may frequently contaminate carcass meats (Stern, 1981). Subsequently, if the meat is improperly processed or if post-processing contamination does occur, the final meat product may contain the human enteropathogen and cause disease in man. Several reports have implicated red meats and poultry as vehicles in its transmission. Raw hamburger (steak tartare), in the culinary traditions of Holland, was consumed in a Dutch military camp, with an explosive outbreak of campylobacteriosis resulting (Oosterom, 1981). The outbreak was epidemiologically traced to the consumption of this food product. Other cases have now implicated both red meats (Park et al., 1982; Peel and McIntosh, 1978) and poultry products (Brouwer et al., 1979) as sources for *Campylobacter* infection.

C. jejuni is a microaerophile, with optimum growth under an atmosphere containing 5% oxygen, 10% carbon dioxide and 85% nitrogen (Kiggins and Plastridge, 1956). Although this precise mixture of gases is not likely to be found in packaged meat systems, it is likely that atmospheres either more or less conducive to the survival of the organism could be encountered. After the use of modified atmospheres and selective packaging, subsequent metabolic activities of both indigenous microflora and meat catabolism occur and alter the gas makeup (Ingram, 1962). The use of vacuum packaging and oxygen impermeable wraps to market meat products has been increasing (Christenson, 1979). Therefore, the objectives of this study were to determine whether this technology might enhance survival of *Campylobacter* and to establish the potential role of vacuum packaging and modified gas atmospheres on the viability of *C. jejuni* in meat products during product storage.

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MATERIALS & METHODS

Cultures, inoculation, and sample storage

The following strains of *Campylobacter jejuni* were employed in this study: USN 509 (human clinical isolate from J.C. Coolbaugh, Naval Medical Research Institute, Bethesda, MD); ATCC 29428 (American Type Culture Collection, Rockville, MD); MGC-2, MGC-4 and 7AC (chicken isolates; USDA, Agricultural Research Service, Meat Science Research Laboratory stock culture collection). Inocula for the storage studies were prepared by transferring 1.0 mL of the stock cultures, maintained in Fluid Thioglycollate medium (Difco, Detroit, MI), to screw-capped test tubes containing 10 mL of Brucella broth (Oxoid, Columbia, MD) with 0.1% agar, which were incubated overnight at 42°C. A 2.0 mL portion from each of the five strains was combined to formulate a mixed culture of 10 mL. The inoculum was added to 10 L of 0.1% peptone (Difco) to obtain a final concentration of approximately 10^5 colony forming units per mL.

Fresh broiler carcasses were purchased from local retail outlets and returned to the laboratory on ice. Upon arrival at the laboratory, the chickens were split in half, using clean, disposable gloves, and the halves were placed in the above prepared inoculating bath for 3 min. The halves were then removed and allowed to drip for an additional 5 min. Subsequently, one half-carcass was placed on a 2S plastic tray (A & E Plastics, Canadaigua, NY) and overlayed with a permeable polyvinyl chloride (PVC) film (PVC Alcoa film, Pittsburgh, PA). The other half-carcass was placed in a standard closure Cry-O-Vac bag and evacuated using a Multivac Model 3696/24 vacuum packager. The samples were held at 4°C and sampled at 48 and 96 hr. At each sampling interval, the accumulated drip from the carcasses and surface rinse were quantitatively assayed for *C. jejuni*, by spread plating onto Campy BAP medium (Blaser et al., 1979), and for the associated aerobic plate counts (APC).

Healthy pigs, sheep and cattle were slaughtered, skinned and eviscerated using conventional methods at the USDA-ARS Beltsville Agricultural Research Center (Abattoir, Beltsville, MD). Flank samples of the animals were removed prior to carcass rinsing and were assayed for the presence of *Campylobacter*. Paired samples were obtained from the flank region from the point of insertion anterior to the stifle joint to a point approximately 15 cm (6 in.) from the proximal location. These regions were sampled because (1) the flank region is thought to be relatively contaminated by feces (Gill and Harris, 1982) and (2) to minimize carcass disfiguration. These flank samples were randomly assigned to vacuum or PVC packaging systems as described above. After 1 wk storage at 2.5°C the samples were rinsed with 250 mL of Cary Blair medium without agar (CB), the rinsings filtered through a double layer of cheesecloth, centrifuged (10 min, 16,000 × *g*) and the pellet was resuspended in 5 mL of CB for both direct plating onto Campy-BAP plates and for enrichment cultures as described by Doyle and Roman (1982).

Bacterial enumeration

Surface counts. The broiler chicken half-carcasses were rinsed with 250 mL of CB. The rinsings were then filtered through cheesecloth

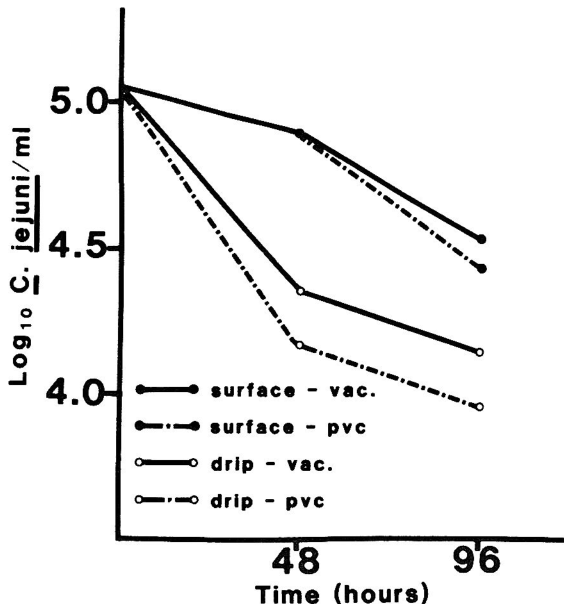

Fig. 1 — Recovery rate of inoculated Campylobacter jejuni *from vacuum packaged and oxygen permeable packaged chickens.*

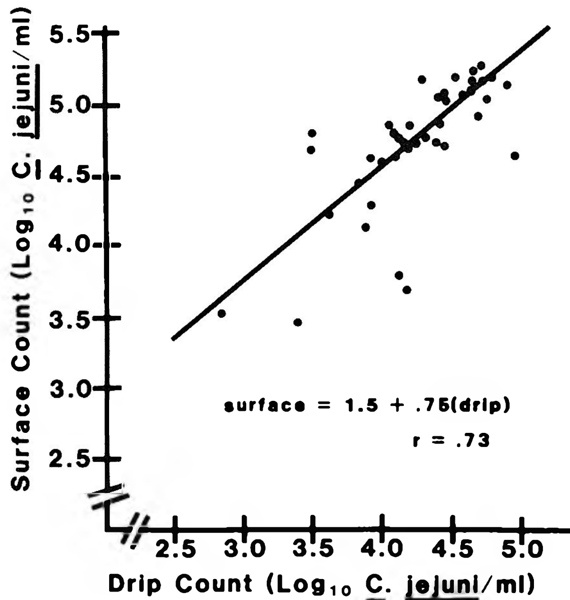

Fig. 2 — Correlations in recovery of inoculated Campylobacter jejuni *by a surface rinse with a drip sampling technique of vacuum packaged chickens stored at 4°C for 96 hr.*

and centrifuged at 16,000 × *g* for 10 min at 4°C. The supernatant was decanted, and the pellet containing the representative microflora was resuspended in 5 mL of CB. The cultures were serially diluted in CB and spread plated onto Campy-BAP medium (Blaser et al., 1979). Plates were incubated at 42°C in an atmosphere of 5% oxygen, 10% carbon dioxide and 85% nitrogen (microaerobic conditions) for 48 hr. Colonies typical of *C. jejuni* were counted, and several representative colonies were prepared as wet mounts for examination using phase contrast microscopy to verify characteristic morphology and motion.

Drip counts. The accumulated drip was aseptically collected from each package with a sterile pipette. The drip was filtered, centrifuged, resuspended and enumerated in the same manner as with the surface rinse determinations.

Aerobic plate counts. Counts were determined by serially diluting the above described bacterial pellet and plating onto Trypticase Soy agar (BBL; Cockeysville, MD) and incubating at 25°C for 48 hr.

Modified gaseous environments

Frozen ground beef was obtained and portions of the meat to be inoculated were screened for *C. jejuni* by selective enrichment (Doyle and Roman, 1982). Prior to inoculating, the ground beef was allowed to thaw for 24 hr at 4°C. A mixture of the five previously listed strains was prepared as indicated above. The mixture was inoculated by using a Hobart Food Cutter Model 84141 to provide uniform level of approximately 10^5 colony forming units per gram. The inoculated 25-g samples were dispensed into Whirlpak bags which were left open in an anaerobic jar (Oxoid, Columbia, MD). The ambient atmosphere was replaced by evacuation and replacement with one of the following: (a) 5% oxygen, 10% carbon dioxide and 85% nitrogen; (b) vacuum of approximately −0.6 bar atmospheres; (c) 100% nitrogen; or (d) 80% carbon dioxide and 20% nitrogen. The anerobic jars were stored at 4°C for a 2-wk period. At each sampling interval (4, 7 and 14 days), a Whirlpak bag was removed and the 25-g sample was mixed with 225 mL of 0.1% peptone by stomaching for 30 sec (Seward Lab-Blender 400; London, England). The homogenated slurries were serially diluted using 0.1% peptone and were spread plated in duplicate onto Campy-BAP agar plates to provide quantitative comparisons.

RESULTS & DISCUSSION

Packaging methods and survival of inoculated *C. jejuni*

No clear differences were observed in the survival of *Campylobacter* as influenced by PVC or vacuum packaging procedures (Fig. 1). The quantitative influence of sampling procedures is also illustrated in Fig. 1 and indicates that declines in the numbers of the organism were parallel in both packaging systems. However, differences were observed in the numbers obtained by the surface rinse method as compared to the drip sampling method. The surface rinse method yielded significantly higher numbers ($P<0.01$) when compared with the drip sample method. On a per carcass-half basis, the drip from vacuum packaged samples manifested a reduction of *C. jejuni* numbers of log 0.91 while the surface rinse from vacuum packaged samples showed a decrease of log 0.52. In the PVC wrapped carcass halves, numbers of the organism in the drip samples were reduced by Log 1.01 while the surface rinse samples had reductions of log 0.62.

Table 1—Isolation of C. jejuni *from red meat carcass samples and from vacuum packaged or polyvinyl chloride (PVC) wrapped meats at 2.5°C for 1 wk*

Livestock species	No. of isolations/ carcasses sampled	No. of isolations/meat samples held at 2.5°C for 1 wk	
		Vacuum	PVC
Porcine	10/27 (37%)	10/27 (37%)	4/27 (15%)
Ovine	10/36 (28%)	9/36 (25%)	3/36 (8%)
Bovine	0/26 (0%)	not done	not done

Sampling method

Further correlations were developed after comparing recovery rates of *C. jejuni* by surface rinse and drip sampling methods. Figure 2 illustrates comparisons of the log number of *C. jejuni* recovered from surface and drip samples of the vacuum packaged chicken. The positive correlation coefficient of 0.73 indicated that the drip may be used to furnish a good index of the relative presence of the organism. A similar correlation of 0.66 was observed using the PVC wrapped product (data not shown). Within the limits of experimental error, and, at the levels of *C. jejuni* typically associated with chickens, the drip sampling technique provides a good, nondestructive sampling approach for assessing the relative presence of *C. jejuni*. Previous work has shown that the bacteriological status of ready to eat poultry can be estimated by drip sample analysis (Mercuri and Kotula, 1964).

Packaging and recovery of indigenous *C. jejuni* and *C. coli*

Table 1 represents the recovery of *C. jejuni* and *C. coli* from pork, lamb, and beef carcasses made through cultural enrichment procedures. The recovery rates of *Campylobacter* from

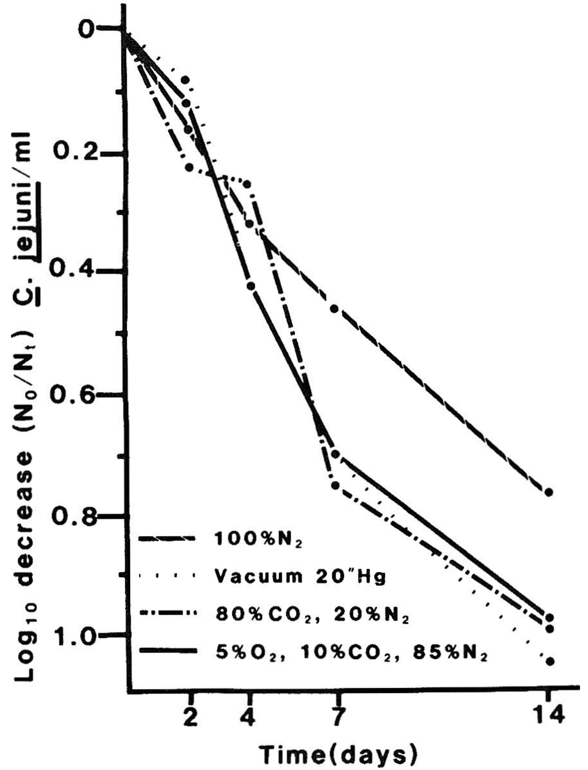

Fig. 3 — Influence of selected gaseous environments on the survival of a mixed culture of Campylobacter jejuni *inoculated into ground beef and stored at 4°C.*

surfaces of freshly slaughtered animals were very similar to previous observations (Stern, 1981). Incidence of *Campylobacter* recovery was fairly consistent among the initially positive samples and those held under vacuum packaging at refrigeration temperatures for 1 wk. The corresponding samples held under PVC packaging at refrigeration temperatures for 1 wk yielded fewer isolations of the organism. These differences attributed to the packaging methods were not consistent with the data previously noted, as no significant difference was observed in the inoculated chicken samples held under the different packaging conditions. Water activities are related to the transmission rates in the packaging materials. The packaging materials had different vapor transmission rates, and this might explain the differences in survival of *Campylobacter*. Also, the longer period of storage (7 days compared with 4 days) might account for some of these differences noted.

Modified atmospheres

Comparisons of the survival responses of *C. jejuni* inoculated into ground beef held under various atmospheric conditions were made in relationship to the control atmosphere of 5% oxygen, 10% carbon dioxide and 85% nitrogen. Results of the survival response of *C. jejuni* are represented in Fig. 3. Statistical analysis indicated that the survival of the organism in ground beef was significantly better ($P<0.01$) in an atmosphere of 100% nitrogen. The rates of inactivation, in terms of D-value, were 21.9 days for the 100% nitrogen, 14.4 days for the control, 13.2 days for the vacuum and 14.0 days for the 80% carbon dioxide and 20% nitrogen atmospheres. The enhanced survival of *C. jejuni* observed in the 100% nitrogen atmosphere corroborates the work of Koidis and Doyle (1983). Hänninen et al. (1984) have previously reported no significant difference in survival of the organism inoculated onto fresh beef and held under (a) vacuum, (b) 20% carbon dioxide and 80% nitrogen, and (c) an atmosphere of 5% oxygen, 10% carbon dioxide and 85% nitrogen.

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Optimization of Extracellular Lipase Activity from *Pseudomonas fluorescens* using a Super-Simplex Optimization Program

G. J. PAQUETTE and R. C. McKELLAR

ABSTRACT

A method of determining extracellular lipase activity from *Pseudomonas fluorescens* based on the hydrolysis of β-naphthyl caprylate has been developed. Optimum activity was determined as a function of temperature, pH, β-naphthyl caprylate and sodium taurocholate concentrations using a stepwise super-simplex optimization procedure. Optimization was performed with a Sharp PC-1500A computer and appropriate ranges for each experimental factor were determined by prior experimentation. A maximum A_{540} response of 0.38 units was obtained at 50°C, pH 7.2, 0.1 mM β-naphthyl caprylate and 17.6 mM sodium taurocholate. This response was 61% better than a previous response obtained by the one-factor-at-a-time method.

INTRODUCTION

EXTRACELLULAR heat-resistant lipases produced by psychrotrophic bacteria may have adverse effects on the quality of milk and other dairy products (Law et al., 1976; Andersson et al., 1981; Adams and Brawley, 1981). Law (1979) has emphasized the need for rapid, simple tests for the presence of bacterial enzymes in milk products; however, little progress has been made towards the development of such a test for bacterial lipases. Roy (1980) and Stead (1983, 1984a, 1984b) have proposed a fluorimetric method based on the release of the fluorescent compound 4-methylumbelliferone from the non-fluorescent ester, 4-methylumbelliferyl oleate.

Recently, a simple colorimetric lipase assay was developed in our laboratory (McKellar, 1986). This method is based on the formation of a colored azo dye by the reaction of a diazonium salt with β-naphthol released from the colorless ester, β-naphthyl caprylate (β-NC), by lipase action. In a previous study, maximum activity was determined by varying experimental conditions independently; however, interactions between factors could not be assessed by this method (McKellar, 1986).

Simplex optimization techniques are useful in improving research efficiency when more than two factors are simultaneously changing during experiments, especially when factors are interacting. Morgan and Deming (1974) have pointed out the value of simplex optimization procedures in analytical chemistry. The basic principle of simplex optimization is to move away from the experiment which has given the worst response in a simplex of N+1 experiments when N is the number of experimental factors. Recently, Nakai et al. (1984) modified the super-simplex optimization procedure of Routh et al. (1977) to avoid stalling at the boundary set as constraint. The purpose of this study was to use simplex optimization to find the levels of the four factors, temperature, β-NC, bile salts and pH required for maximum extracellular lipase from *Pseudomonas fluorescens* B52.

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MATERIALS & METHODS

Preparation of lipase

Pseudomonas fluorescens strain B52 (obtained from Dr. B.C. Richardson, New Zealand Dairy Research Institute) was cultured in pyruvate mineral salts medium (McKellar and Cholette, 1984) at 20°C with shaking at 180 rpm for 48 hr. Cells were removed by centrifugation (5000 × *g* for 10 min) and the supernatant stored at 4°C and used as the source of crude lipase.

Lipase assay

The assay was performed as described previously (McKellar, 1986). The assay mixture contained, in a final volume of 2.0 mL: 50 mM buffer pH 5.5–10.0; 0.1–2.0 mM β-NC (Sigma Chemical Co., St. Louis, MO); 0–20.0 mM sodium taurocholate (NaTC; Sigma) and an appropriate dilution of enzyme. Buffers employed (all from Sigma) were; 2[N-morpholino]ethane sulfonic acid (MES) pH 5.5–6.5; N,N-bis[2-hydroxyethyl]-2-aminoethane sulfonic acid (BES) pH 6.5–7.8; and 2[N-cyclohexylamino]ethane sulfonic acid (CHES) pH 8.6–10.0.

Triplicate 15 mL conical centrifuge tubes containing the reaction mixture were incubated at 20–50°C for 30 min. Fast Blue BB salt (Sigma; 0.02 mL of a freshly prepared 100 mM solution in dimethyl sulfoxide) was added and after incubation at 35°C for 5 min, the reaction was terminated by the addition of 0.2 mL 0.72N trichloroacetic acid. The insoluble azo dye was extracted by vigorous mixing with 5 mL ethyl acetate and after centrifugation at 5000 × *g* for 10 min, the absorbance at 540 nm of the upper layer was determined on a Bausch and Lomb Spectronic 21 (Fisher Scientific Co., Fairlawn, NJ). Controls lacking enzyme were used as blanks.

Super-simplex optimization

Super-simplex optimization was performed as described by Nakai et al. (1984) using a program written for the Sharp PC-1500A computer. The program selected a Starting Simplex of five experiments with levels of each of temperature, pH, β-NC and NaTC within the ranges given above. Responses from these experiments were then used by the program to develop a series of sequential experiments (Iterative Steps) leading to the optimum response (A_{540} units). Controls utilizing the conditions of experiment (vertex) 2 were performed with each of the Iterative Steps starting with vertex 6 and results were normalized to the initial response obtained with vertex 2. This was carried out to correct for day-to-day variations in experimental conditions. Vertex 2 was selected as the experiment having the best response in the Starting Simplex. Mapping was performed for NaTC after 25 experiments as described by Nakai et al. (1984).

RESULTS

A PROGRESSIVE INCREASE in enzyme activity towards the optimum response is demonstrated in Fig. 1. Maximum enzyme activity was achieved at vertex 19, with slightly lower responses observed at vertex 11 and 15. Significant decreases in activity were noted at vertex 12 and 16, followed by steady increases indicating that the program was attempting to locate other optima by making major changes in factor levels. Figure 1 also shows that the response approached the optimum rapidly during initial experiments, but that later experiments served only to make minor adjustments in the response, in agreement with the observations of Nakai et al. (1984).

Boundary values (i.e., upper or lower ranges set during the Initial Simplex) for temperature, NaTC and β-NC were at-

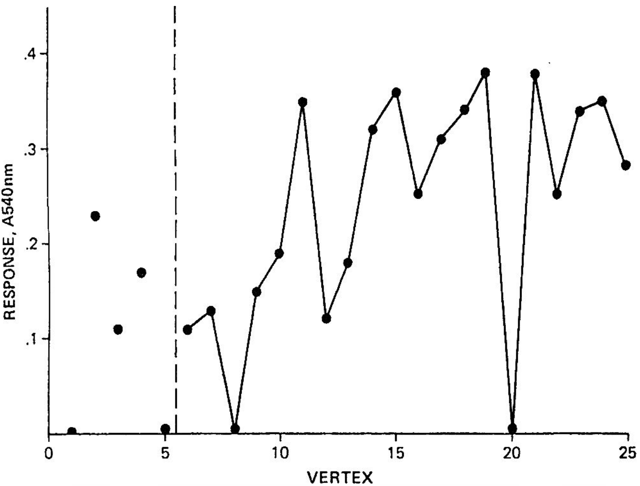

Fig. 1—Measured experimental response (absorption at 540 nm) as a function of vertex (sequential experiment).

tained at vertex 11 and 15 (Fig. 2A, B, and C). Near-optimal pH values were also selected at vertex 10 and 11, and further changes in pH were slight (Fig. 2D). Optimum activity was noted at vertex 19 (Fig. 1); however, stalling at the boundaries may have prevented attainment of the true optimum. This limitation can be overcome by allowing the search to freely violate boundary constraints when selecting conditions for subsequent experiments. This resulted in selection of markedly different conditions at vertex 20 (including a negative β-NC concentration; Fig. 2B). Since a negative substrate concentration selected by the program was unrealistic, the concentration was taken to be zero. After a response of zero at vertex 20 (Fig. 1), an optimal response was again achieved at vertex 21 and further experimentation failed to improve the response. At this point, the optimization was considered complete.

The relationship between response values and factor range is shown in Fig. 3. Best response values were associated with narrow temperature (Fig. 3A) and β-NC (Fig. 3B) ranges, concentrated close to upper and lower boundaries respectively. Higher activity was associated with a fairly narrow pH range (Fig. 3D); however, variations in NaTC concentration appeared to have only slight effect on activity (Fig. 3C). In this case, response values greater than 0.25 were associated with NaTC concentrations of 13 to 20 mM.

The mapping option of super-simplex optimization (Nakai et al. 1984) was carried out in order to arrive at a more precise optimum for NaTC concentration. Fig 3C shows that factor level trends were moving toward an optimum at approximately 20.0 mM NaTC.

Conditions from vertex 19 were selected for comparison with optimal conditions determined in a previous study (McKellar, 1986). Table 1 shows that the experimental conditions selected in the present study differ considerably from those obtained earlier; a significantly higher temperature and NaTC concentration were selected by the program, while the optimum β-NC concentration was 10-fold lower than was pre-

Fig. 2—Variations in factor levels as a function of vertex (sequential experiment): (A) temperature; (B) β-naphthyl caprylate; (C) sodium taurocholate; (D) pH.

Table 1—Experimental conditions for optimal lipase activity determined by manual and super-simplex optimization procedures

Method	Temp (°C)	β-NC (mM)	NaTC (mM)	pH	Activity (μmol/mL/hr)	% Relative activity
Manual[a]	40	1.0	6	8.0	32.9	100
Super-simplex	50	0.1	17.6	7.2	53.0	161

[a] McKellar (1986)

viously found. The optimum pH of 7.2 was only slightly less than the pH of 8.0 selected by manual optimization. An increase in activity of 61% was realized by the use of super-simplex optimization.

DISCUSSION

THE PRESENT STUDY has clearly demonstrated the advantages of a computer-directed optimization procedure for maximizing enzyme activity. The method is simple and may be applied to a variety of food-related situations including optimization of assays for milk quality assessment and selection of best processing conditions for the manufacture of a variety of products. The main disadvantage of the sequential methodology, e.g., simplex optimization, is the time required to perform sequential experiments, especially when individual experiments may require one or more days to perform; however, the method requires little input from the experimenter, thus the entire procedure may be carried out by a technician without reference to his supervisor.

The results demonstrated the value of using an objective method for optimization; interaction between factors was taken into account and experimental bias was reduced. Thus, experimental conditions which may not be considered feasible by the experimenter may be selected. For example, in a previous study (McKellar, 1986), a significant loss of activity was noted at temperatures above 40°C. Selection of a higher (50°C) temperature during super-simplex optimization may have resulted from a protective effect exerted by NaTC towards the enzyme.

Nakai et al. (1984) have introduced a mapping step in the super-simplex optimization procedure. This option is useful when response values are widely scattered and the optimum is difficult to locate. Points in a response vs factor level scatter diagram are linked as determined by the program, based on data grouping by factor obtained under approximately the same experimental conditions. When applied to NaTC, this procedure indicated that the true optimum lay in the region of 20 mM of the bile salt. Although mapping was of some value in the present situation, Nakai et al. (1984) have pointed out that

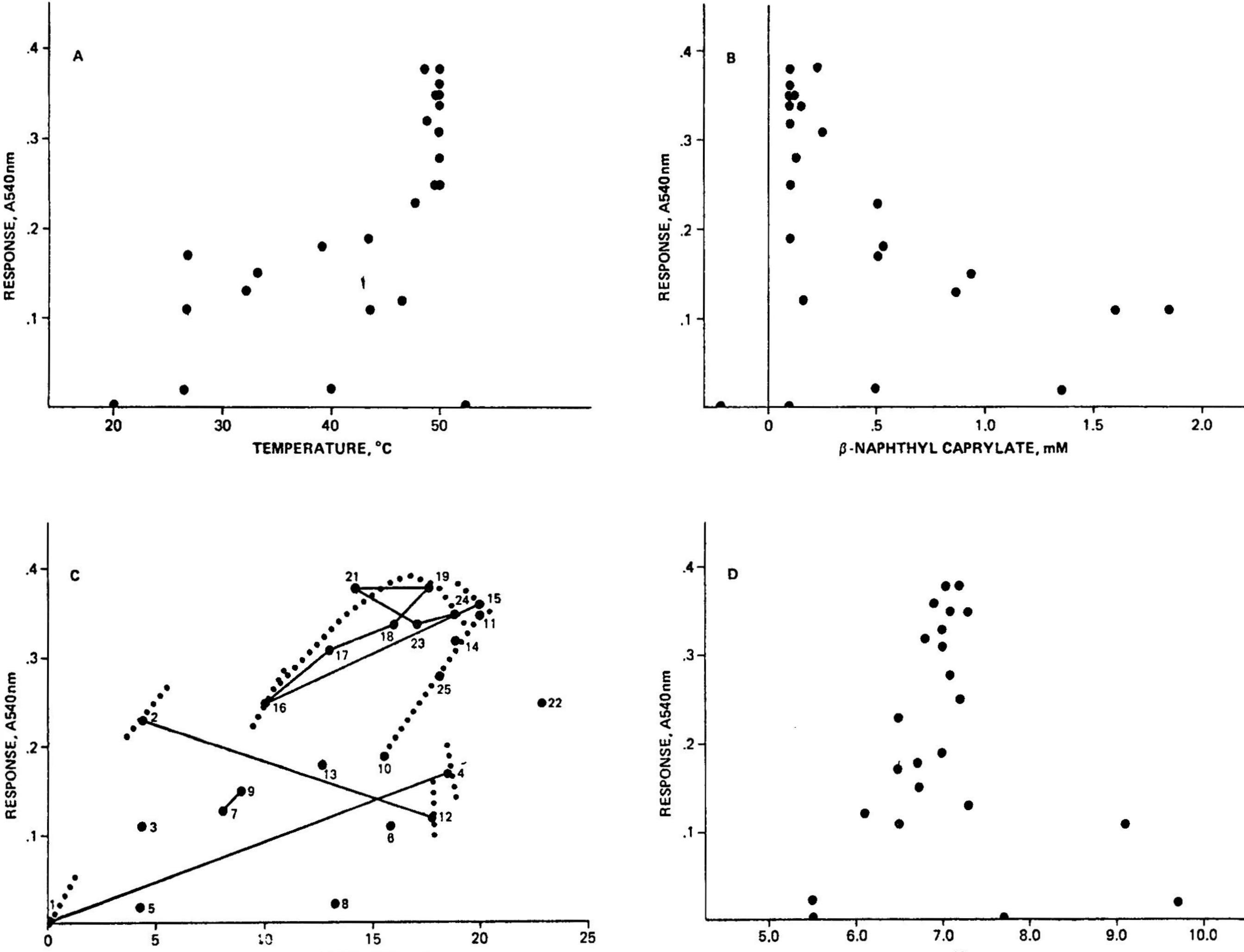

Fig 3—Measured experimental response as a function of factor level: (A) temperature; (B) β-naphthyl caprylate; (C) sodium taurocholate (NaTC); (D) pH. Solid lines and vertex numbers were obtained by the mapping option of super-simplex optimization. Broken lines indicate progression towards an optimum at 20.0 mM NaTC.

this option is seldom required in most food science applications.

In the present study, some difficulty was experienced when factor levels approached boundaries. Immediate removal of constraints may have reduced the number of experiments necessary to obtain the optimum response; however, boundaries are often dictated by practical considerations. In the present study, use of NaTC in excess of 20 mM significantly increased the viscosity of the assay mixture making extraction difficult. In addition, it would be impractical to employ temperatures above 50°C since many psychrotroph lipases encountered in milk are subject to heat inactivation at temperatures between 50° and 60°C (Griffiths et al. 1981).

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SUGAR COOKIES WITH LUPINE FLOUR. . .From page 646

Protein efficiency ratio

The rat study showed that the animals consuming the control cookies gained practically no weight during the test period and the PER value was 0.15 ± 0.25. One may speculate that this value was due to the low level of dietary protein (6.5%) provided by wheat flour and to protein damage caused by heat during the baking process. The rats consuming the FFSL cookies gained significantly ($p<0.01$) more weight as the levels of FFSL increased from 14.0 g ± 4.0 to 49.0 ± 3.5 for 5 and 25% FFSL, respectively. PERs values also were significantly higher ($p<0.001$) than the control cookies reaching a plateau for the cookies containing 20% FFSL, PER 1.81 ± 0.13 (Table 5).

Several studies have been conducted incorporating sweet lupine in such popular foods as spaghetti (Gardiman and Ballester, 1984), milk substitutes (Ivanović et al., 1983) and cream soups (Oliva and Ballester, 1984). In all of them their nutritional quality was increased and they had good acceptability among adults and children.

The results of the present investigation open a new way of using sweet lupine flour in the human diet. Presently, sensory evaluation and acceptability tests of FFSL cookies in children are being conducted and the results will be reported.

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Biotransformation of Linalool by *Botrytis cinerea*

G. BOCK, I. BENDA, and P. SCHREIER

ABSTRACT

Biotransformation of linalool was studied using grape must and three strains of *Botrytis cinerea* (5901/2; 5909/1; 5899/4). Capillary gas chromatography and combined capillary gas chromatography-mass spectrometry revealed predominant conversion (>90%) of linalool to (E)-2,6-dimethyl-2,7-octadiene-1,6-diol. In minor concentrations (<10%), the corresponding (Z)-isomer, 2-vinyl-2-methyl-tetrahydrofuran-5-one, the four (E)-and (Z)-linalool oxides in their furanoid and pyranoid forms, the (E)- and (Z)-acetates of pyranoid linalool oxides as well as 3,9-epoxy-p-menth-1-ene were identified.

INTRODUCTION

ALCOHOLIC BEVERAGES such as wine and beer are classical examples of biotechnological production of foods. During their processing, various enzyme activities of yeasts catalyze the biotransformation of plant constituents leading to a complex mixture of flavor compounds, which determine, in a characteristic quantitative distribution, the quality of the final product (Schreier, 1984). In winemaking, in certain cases the influence of a fungus, i.e. *Botrytis cinerea*, also has to be considered (Dittrich, 1977; Krogh and Carlton, 1982). In an early, unripe state of maturation, the infection of grapes by *B. cinerea* is feared, as the grapes become moldy. With fully ripe grapes, however, the growth of *B. cinerea* is promoted, as grapes infected by the ''noble rot'' deliver the famous sweet wines, such as Sauternes of France, Tokay Aszu of Hungary, or Trockenbeerenauslese wines of Germany.

The high metabolic activity of *B. cinerea* is well-known. Thus, the formation of glycerol and gluconic acid as well as citric acid in grape musts infected by *B. cinerea* has been observed (Bertrand et al., 1976; Dittrich, 1977). As to the volatiles of grapes and wines, some years ago, several key components were identified by our group (Schreier et al., 1976; Schreier, 1984) consisting of terpenes and derived terpenoids. As some of these compounds, including terpene alcohols such as, e.g., linalool, have been found to be affected by *B. cinerea*, in the course of our work on microbial transformation of volatiles we dealt with the metabolization of terpene alcohols by this fungus. It was the aim of this study to identify the structures of fungal metabolization products, which had not been characterized in the previous work (Boidron, 1978; Boidron and Torrès, 1978; Shimizu et al., 1982).

MATERIALS & METHODS

Botrytis cinerea strains

The *B. cinerea* strains 5901/2, 5901/1, and 5899/4, used in this study, were obtained from the collection of the Bayerische Landesanstalt für Weinbau und Gartenbau, Würzburg. From the original cultures, a part was transferred to malt agar slants and incubated at 25°C for 7 days.

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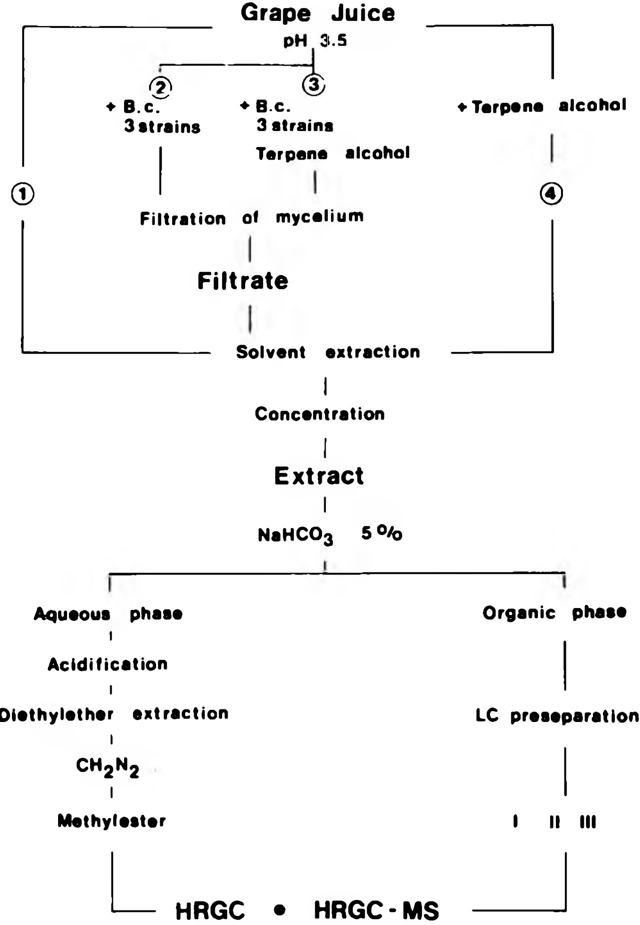

Fig. 1—Scheme of sample preparation steps. 1-grape juice volatiles; 2-botrytized musts (3 strains, 5901/2; 5909/1; 5899/4); 3-addition of linalool to the botrytized musts; 4-addition of linalool to the must. B.c. = B. cinerea.

Incubation conditions

The sugar and acid content of the grape must (cultivar, Müller-Thurgau) used were adjusted to 200 g/L and 8.5 g/L (= pH 3.5), respectively. After sterilization (30 min at 110°C) the grape must (700 mL) was filled into 1 L-Erlenmeyer flasks using 0.2 μm membrane filter. Each flask was inoculated with a pure *B. cinerea* strain and incubated at 25°C for 2 wk. The mycelium was removed and the solutions analyzed by capillary gas chromatography (HRGC) and combined capillary gas chromatography-mass spectrometry (HRGC-MS). In like manner, blank tests without *B. cinerea* incubation, and experiments after addition of 50 mg/L linalool to the must with and without *B. cinerea* incubation were carried out (cf. Fig. 1). Additionally, in two control experiments, 2,6-dimethyl-3,7-octadiene-2,6-diol and 2,6-dimethyl-1,7-octadiene-3,6-diol were added (10 mg/L) to the botrytized must. All steps were performed under strictly sterile conditions.

Isolation of volatiles and preseparation

After addition of internal standards (ethyl (E)-2-butenoate, 0.40 mg/L; 4-methyl-1-pentanol, 0.49 mg/L) to the untreated and botrytized musts solvent extraction was carried out using a pentane-dichloro-

Table 1—Mass spectrometric data of linalool biotransformation products

Compound	MS data m/z (%)[a]			
(E)-2,6-dimethyl-2,7-octadiene-1,6-diol (19)	43(100)	71(41)	67(38)	41(28)
	55(26)	68(15)	82(6)	81(4)
(Z)-2,6-dimethyl-2,7-octadiene-1,6-diol (20)	43(100)	71(51)	67(51)	41(36)
	55(30)	68(20)	82(12)	81(8)
(Z)-linalool oxide, furanoid (21)	43(25)	55(20)	59(100)	67(20)
	68(25)	93(25)	94(35)	111(5)
(E)-linalool oxide, furanoid (22)	cf. (Z)-isomer			
(Z)-linalool oxide, pyranoid (23)	41(26)	43(50)	59(80)	87(53)
	68(100)	79(20)	78(19)	94(63)
(E)-linalool oxide, pyranoid (24)	cf. (Z)-isomer			
(Z)-linalool oxide acetate, pyranoid (25)	41(10)	43(100)	55(9)	59(11)
	67(8)	68(17)	93(8)	94(29)
(E)-linalool oxide acetate, pyranoid (26)	cf. (Z)-isomer			
3,9-epoxy-p-menth-1-ene (27)	137(100)	69(50)	41(37)	109(30)
	79(31)	91(15)	55(14)	93(12)
2-vinyl-2-methyl-tetrahydrofuran-5-one (28)	27(76)	41(35)	43(95)	55(78)
	56(34)	67(52)	71(40)	111(100)

[a] The eight most intense peaks are represented.

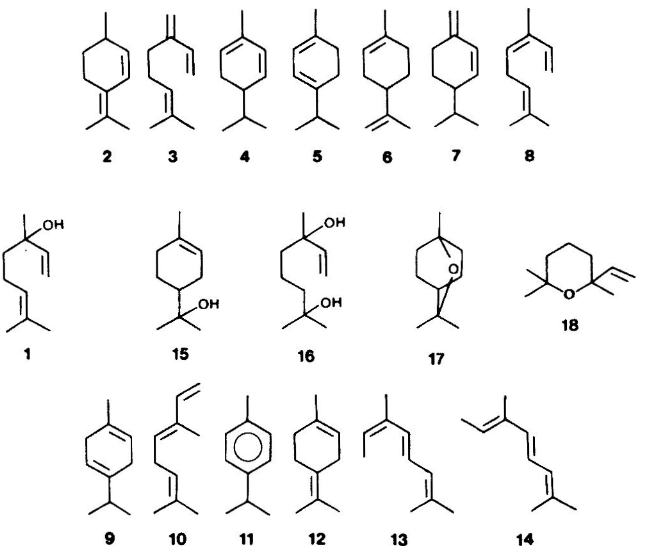

Fig. 2—Structures of terpenoids chemically formed from linalool (1) under acidic conditions (experiment 1, Fig. 1). (2) 2,4(8)-p-menthadiene; (3) β-myrcene; (4) α-phellandrene; (5) α-terpinene; (6) limonene; (7) β-phellandrene; (8) (Z)-ocimene; (9) γ-terpinene; (10) (E)-ocimene; (11) p-cymene; (12) terpinolene; (13) (E,Z)-alloocimene; (14) (E,E)-alloocimene; (15) α-terpineol; (16) 3,7-dimethyl-1-octene-3,7-diol; (17) 1,8-cineole; (18) 2,2,6-trimethyl-2-vinyl-tetrahydropyran.

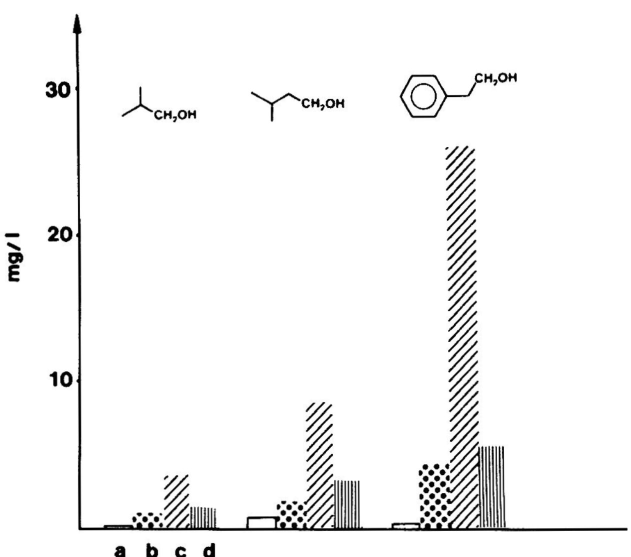

Fig. 3—Formation of 2-methyl-1-propanol, 3-methyl-1-butanol and 2-phenylethanol by three strains of B. cinerea (a = grape must; b = 5901/2; c = 5909/1; d = 5899/4).

methane mixture (2:1) as described by Drawert and Rapp (1968). Acids were removed from the extracts by separation with 5% $NaHCO_3$ solution, and neutral volatiles were carefully concentrated to 1 mL using a Vigreux column (45°C). The concentrates were preseparated into fractions of different polarity by adsorption chromatography on silica gel with a pentane-diethyl ether gradient (60 mL/hr) (Idstein and Schreier, 1985). Fraction I: 200 mL pentane; fraction II: 200 mL pentane + diethyl ether (9 + 1 v/v); fraction III: 200 mL diethyl ether. The eluates were dried over anhydrous sodium sulfate and carefully concentrated using a Vigreux column (45°C) to 0.1 mL for HRGC and HRGC-MS analysis.

Capillary gas chromatography (HRGC)

Instrument: Carlo Erba Fractovap 4160 fitted with a flame ionization detector (FID) and an air-cooled on-column injector. Column: J & W DB-Wax (30 m × 0.32 mm i.d.; df = 0.25 μm) fused silica capillary, connected with a 2m uncoated fused silica precolumn ("retention gap") (Grob and Müller, 1982). On-column injection was used. The temperature program was isothermal for 2 min at 50°C, then 50° to 240°C at 5°C/min. The flow rates for the carrier gas were 2 mL/min He, for the make-up gas 30 mL/min N_2 as well as for the detector gases 30 mL/min H_2 and 300 mL/min air, respectively. The detector temperature was kept at 220°C. Volumes of 0.3 μL were injected.

Results of qualitative analyses were verified by comparison of HRGC retention and mass spectral data with those of authentic reference substances. Quantitative determinations were carried out by standard controlled calculations using a Hewlett-Packard 3388 A laboratory data system considering extraction yields and HRGC response factors.

Capillary gas chromatography-mass spectrometry

Instrument: Finnigan MAT 44 quadrupole mass spectrometer coupled by an open-split interface with a Varian Aerograph 1440 equipped with a water-cooled on-column injector. A J & W DB-Wax (30 m × 0.32 mm i.d.; df = 0.25 μm) fused silica capillary column connected to a 2m uncoated piece of fused silica capillary column as the "retention gap" (Grob and Müller, 1982) was used. The conditions were as follows: temperature, isothermal for 5 min at 60°C and then from 60° to 240°C at 5°/min; carrier gas flow rate, 2.5 mL/min He; temperature of ion source and all connection parts, 200°C; electron energy, 70 eV; cathodic current, 0.8 mV; injection volumes, 0.3 μL.

Reference compounds

The preparation of (E)- and (Z)-2,6-dimethyl-2,7-octadiene-1,6-diol was performed by SeO_2 oxidation of linalool according to Behr et al. (1978). The MS data are outlined in Table 1. Syntheses of 2,6-dimethyl-3,7-octadiene-2,6-diol [m/z (%): 43(100)-82(84)-71(70)-67(48)-41(23)-55(16)-85(5)-81(3)] and 2,6-dimethyl-1,7-octadiene-3,6-diol [m/z (%): 43(100)-67(78)-71(62)-55(41)-41(40)-82(36)-68(26)-69(15)]

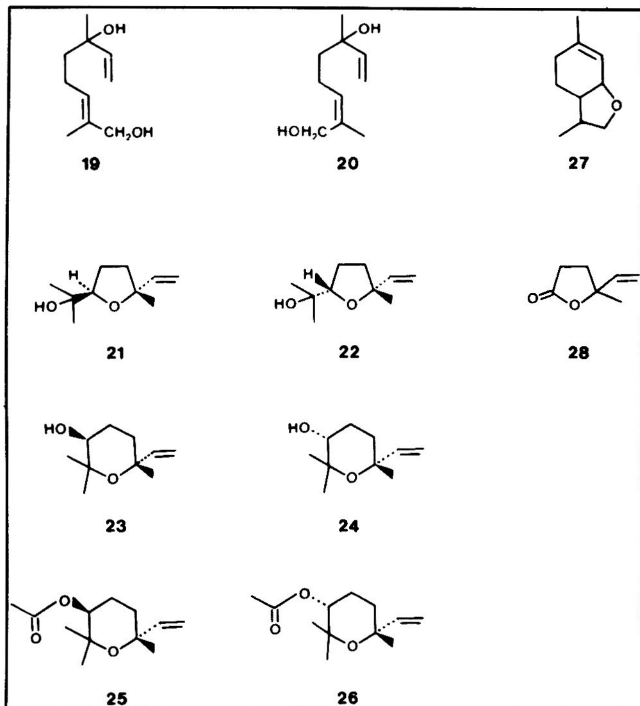

Fig. 4—Structures of terpenoids formed from linalool (1) by B. cinerea. (19),(20) (E)- and (Z)-2,6-dimethyl-2,7-octadiene-1,6-diol; (21),(22) (Z)- and (E)-linalool oxides, furanoid; (23),(24) (Z)- and (E)-linalool oxides, pyranoid; (25),(26) (Z)- and (E)-linalool oxide acetates, pyranoid; (27) 3,9-epoxy-p-menth-1-ene; (28) 2-vinyl-2-methyl-tetrahydrofuran-5-one.

were carried out by photooxygenation of linalool followed by mild reduction (Kjøsen and Liaaen-Jensen, 1973).

RESULTS & DISCUSSION

IN THE LINALOOL control experiment (line 4 in Fig. 1) linalool (1) underwent a variety of well-known chemical reactions [hydrolysis, deprotonation, hydration, cyclization (Morin and Richard, 1985)] leading to a series of hydrocarbons (2)-(14) as well as α-terpineol (15), 3,7-dimethyl-1-octene-3,7-diol (16), 1,8-cineole (17) and 2,6,6-trimethyl-2-vinyl-tetrahydropyran (18) shown in Fig. 2.

When *B. cinerea* was added to the grape must (line 2 in Fig. 1) higher alcohols originating from the amino acid metabolism, such as 2-methyl-1-propanol, 3-methyl-1-butanol and 2-phenylethanol, were found as metabolic products from *B. cinerea* (Fig. 3). There were distinct quantitative differences depending on the strain used. With additional control experiments it could be demonstrated that these alcohols were formed exclusively by *B. cinerea* and not by an eventual contamination by yeasts. This fact has to be stressed as contradictory results have been published (Bertrand et al., 1976; Dittrich, 1977).

After addition of linalool to the botrytized must (line 3 in Fig. 1), a series of transformation products was identified by HRGC and HRGC-MS (Fig. 4). The conversion products comprised (E)- (19) and (Z)-2,6-dimethyl-2,7-octadiene-1,6-diol (20), the furanoid (Z)-(21) and (E)-linalool oxides (22), the pyranoid (Z)- (23) and (E)-linalool oxides (24), the isomer acetates of the latter ones (25), (26), 3,9-epoxy-p-menth-1-ene (27) and 2-vinyl-2-methyl-tetrahydrofuran-5-one (28). The MS data of these fungal conversion products of linalool are presented in Table 1. Quantitative HRGC showed that linalool was predominately (>90%) metabolized to (E)-2,6-dimethyl-2,7-octadiene-1,6-diol (19) by *B. cinerea*; the compounds (20)-(28) were only found as by-products in minor concentrations.

As to the possible biogenetic pathways of metabolic products formed from linalool by *B. cinerea*, the terpene diols 2,6-dimethyl-3,7-octadiene-2,6-diol (29) and 2,6-dimethyl-1,7-octadiene-3,6-diol (30) (Fig. 5), both detected among the natural grape must constituents (Fig. 1, 1), might function as intermediates in the formation of 2,6-dimethyl-2,7-octadiene-1,6-diol (19). However, in control experiments, in which the diols (29) and (30) were added to the botrytized must instead of linalool, no formation of (19) or (20) could be observed. Consequently, for the production of (E)-(19) a direct enzymic ω-hydroxylation of linalool can be considered, as previously proposed for analogous reactions of bacterial metabolization of linalool (Fig. 5) (Devi et al.; 1977; 1978; Madyastha, 1984). Contrary to the above-mentioned odorless diols (29) and (30), 2,6-dimethyl-2,7-octadiene-1,6-diol is an odoriferous compound, useful in perfume and flavor industry (Jap. Pat., 1983).

Fig. 5—Scheme of biogenetic formation of (19) and (20) by direct ω-hydroxylation of linalool (1) and exclusion of diols (29) and (30) as precursors of (19) and (20). Formation of (27) by allylic rearrangement and cyclization from (19) (Kitagawa et al., 1983).

As to the by-products of linalool transformation by *B. cinerea* the formation of 3,9-epoxy-p-menth-1-ene (27), the character impact compound of fresh dill herb (Schreier et al., 1981), can be understood by allylic rearrangement of (E)-2,6-dimethyl-2,7-octadiene-1,6-diol (19) to the corresponding hydroxygeraniol (-nerol), which is known to undergo cyclization to the epoxy derivative (27) under acidic conditions (Kitagawa et al., 1983).

For the formation of isomer hydroxy ethers (21)-(24), the

Fig. 6—Scheme of biogenetic pathways for the formation of isomer linalool oxides (21)-(24) and their pyranoid acetates (25),(26) from linalool (1).

so-called linalool oxides, the diastereoisomers of 6,7-epoxylinalool (31) have been proposed as biogenetic precursors (Fig. 6) (Ohloff et al., 1985). Recently, in our studies of the precursors of papaya fruit volatiles, the two 6,7-epoxy-linalool isomers have been detected as natural constituents of this fruit (Winterhalter et al., 1986). Due to the acidic nature of the medium (pH 3.5) in the present study, the detection of the labile epoxy derivatives (31) could not be expected. From the hydroxy ethers (23) and (24) the formation of acetates is easy to understand.

Finally, the lactone (28) is generally regarded as a formal oxidation product of furanoid linalool oxides (21) and (22), but the biogenetic formation pathway of this compound has not been elucidated as yet.

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Effect of Pressure-Heat Treatments on Cathepsin B1 Activity

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ABSTRACT

The substrate Nα-benzoyl-DL-arginine-2-naphthylamide was used to measure the activity of crude cathepsin B1 in reaction mixtures that had been subjected to various combined pressure and heat treatments. Pressures of 100 kPa (atmospheric), 100 MPa and 150 MPa and temperatures in the range 30–80°C were used. High pressure appeared to protect the enzyme against heat inactivation. Maximum activity was observed at 60°C and 150 MPa and was 12 times that observed at 60°C and atmospheric pressure. An increase in activity of cathepsin B1 may account, at least in part, for the tenderization of meat by pressure-heat treatments.

INTRODUCTION

THE MEAT TENDERIZING EFFECT of the pressure-heat treatment of Bouton et al. (1977a) was attributed to the disaggregation under pressure of some myofibrillar components. However, muscle contains endogenous enzyme systems which are believed to degrade structural proteins in muscle and thus improve meat tenderness (Moeller et al., 1977). Accordingly, increased enzymic action also may be a way by which pressure-heat treatment achieves its tenderizing effect.

Although Locker and Wild (1984) have inferred that catheptic proteases are inhibited by pressure treatment, Horgan (1981) has speculated that cathepsin B was responsible for the breakdown of sarcoplasmic reticulum in muscles held for 10 min at 150 MPa and 35°C. Gonzalez-B (1983) and Elgasim et al. (1983) have claimed that pre-rigor pressure treatment ruptures lysosomal membranes and releases catheptic enzymes that are capable of degrading myofibrillar proteins (Schwartz and Bird, 1977) and connective tissues (Burleigh et al., 1974; Gonzalez-B, 1983). If the release of lysosomal enzymes is enhanced in the pressure-heat treatment, and if some of these proteases are activated by pressure, as are approximately 30% of the enzymes so far examined (Morild, 1981), their contribution to the pressure-heat effect could be significant.

The aim of this investigation was to determine whether cathepsin B1 remained active during pressure-heat treatments and if so, how its activity was affected.

MATERIALS & METHODS

CATHEPSIN B1 was prepared from bovine spleen according to the procedure of Barrett (1973) except that the purification process was terminated immediately prior to the CM-cellulose chromatography step. The solution so obtained was diluted 1 in 50 with deionized water to obtain an enzyme concentration suitable for the assay procedures. The diluted solutions contained 7 units/mL (Barrett, 1972) of cathepsin B1 activity.

The concentrations and ratios of reagents used in the assay of enzyme activity during pressure-heat treatment were according to Barrett (1972), but the volumes were adjusted to give a reaction mixture of 16.4 mL. No pre-incubation heating was performed. The enzyme solution was held at 2°C; the buffer and substrate solutions were held at 25°C. Aliquots of the reagents were mixed rapidly and sealed in two 7 mL reaction bottles. Each bottle was then sealed in a pressure vessel. Both pressure vessels had chamber diameters of 2.54 cm and lengths of 7.6 cm and were located in baths that could be filled with water.

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Experiment 1

Effect of incubation temperature. Pressure was applied in one of the vessels and heated water was immediately added to the baths to rapidly heat the samples to the nominated incubation temperature. This was maintained within ± 0.5°C of the desired temperature by heating units. Incubation temperatures of 30, 40, 50, 60, 70, and 80°C were used in combination with pressures of 100 and 150 MPa. The sample in the nonpressurized (atmospheric pressure) vessel served as the control. Incubation time was taken to be the elapsed time between the application and the release of pressure and for this experiment was 30 min. After the pressure was released, both pressure vessels were rapidly opened and duplicate 2 mL aliquots of the reaction mixtures were withdrawn and mixed with 1 mL aliquots of chloromercuribenzoate solution (Barrett, 1972). Samples were accumulated during a day and their naphthylamine content determined by reaction with a fresh solution of Fast Garnet GBC and reference to a standard curve. This procedure overcame problems associated with the instability of the combined chloromercuribenzoate and Fast Garnet solutions (Barrett, 1972).

Experiment 2

Effect of incubation time. In this experiment the effect on enzyme activity of varying incubation times at 150 MPa and at 30, 60 or 80°C was assessed. Incubation times of 10, 30, and 60 min were used. The reaction mixture was prepared as in experiment 1. A random block design was used whereby two samples were heated for the same time but at different temperatures. At the conclusion of the incubation time the samples were treated as in experiment 1. In both experiments enzyme activity was assessed in terms of nanomoles of naphthylamine released (in the 2 mL aliquot withdrawn).

The effect of leupeptin on enzyme activity was assessed by adding leupeptin at 50 μg/mL to a sample that was incubated at 60°C and 150 MPa for 30 min. The stability of the synthetic substrate Nα-benzoyl-DL-arginine-2 naphthylamide (BANA) was tested by heating the reaction mixture and substrate at 80°C and 150 MPa for 60 min.

Statistical analysis

Experiment 1 was completely randomized with three pressures (atmospheric, six replicates; 100 MPa, three replicates; 150 MPa, three replicates) in a factorial arrangement with six temperatures. Experiment 2 was a randomized block on two days with three temperatures by three times each replicated twice. For both experiments enzyme activity was analyzed by analysis of variance techniques and appropriate LSD values calculated.

RESULTS & DISCUSSION

THE ENZYME ACTIVITY observed in these experiments can be attributed to cathepsin B1 on the grounds of the specific substrate degraded (BANA) and the fact that this degradation was inhibited by leupeptin and chloromercuribenzoate. No free naphthylamine was detected when reaction mixtures free of the enzyme preparation were incubated at 80°C and 150 MPa for 60 min.

The results obtained for 30 min incubation at temperatures between 30 and 80°C (experiment 1) are presented in Fig. 1. The optimum temperature of incubation for the enzyme at atmospheric pressure was 40°C. This corresponds to the tem-

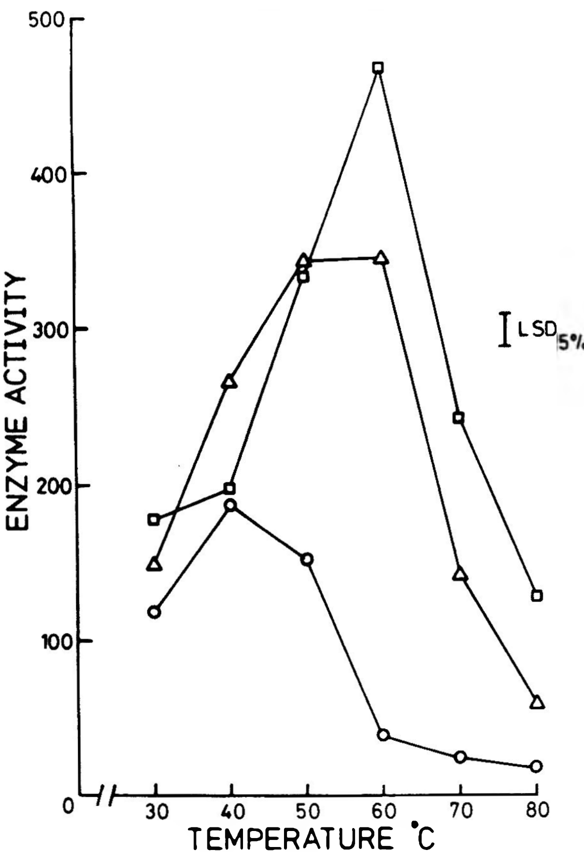

Fig. 1—Effect of incubation temperature on enzyme activity at atmospheric pressure (control), 100 MPa and 150 MPa for an incubation time of 30 min. Activity is expressed at nanomoles of naphthylamine released per 2 mL of reaction mixture. ○—○ 100 kPa; △—△ 100 MPa; □—□ 150 MPa.

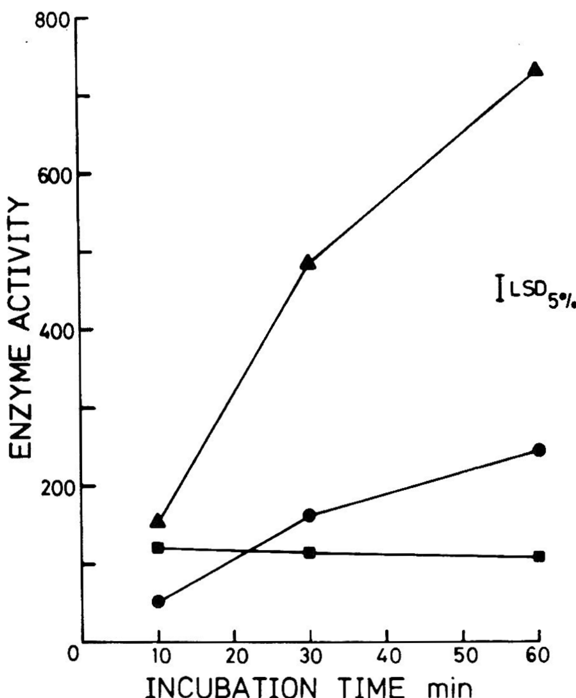

Fig. 2—Effect of incubation time on enzyme activity at three temperatures and 150 MPa. Activity is expressed as nanomoles of naphthylamine released per 2 mL of reaction mixture. ●—● 30°C, ▲—▲ 60°C; ■—■ 80°C.

perature recommended by Barrett (1972) for the assay of cathepsin B1. At temperatures above 40°C, the activity decreased and at 60°C was well below the level at 30°C. This probably indicates thermal denaturation of the enzyme. Rapid inactivation of the enzyme was apparent at 80°C since the amount of product (naphthylamine) formed was not significantly different from that present immediately before the application of heat. Thirteen nanomoles of naphthylamine were formed in the time taken to seal the reaction mixture into the pressure vessel and apply heat. Treatment conditions of 150 MPa at 60°C gave highest activity; at 100 MPa, activity was similar at 50 and 60°C. At 30°C the pressure treated samples showed enhanced enzyme activity compared to the control, and the hydrolysis rate appeared to increase with increasing pressure. However, this simple explanation is confounded by the effect of temperature because at the enzyme's optimum temperature (40°C) the activity was highest at 100 MPa. At high temperatures (60°C and above) where thermal inactivation of the control was apparent, the pressure treated samples showed greater activity although inactivation of the enzyme still occurred at 70 and 80°C. Thus, pressure appeared to have a protective effect on the heat inactivation of cathepsin B1 but temperature acted as a greater stimulator or inhibitor of activity. However, the magnitude of the pressure treatment appeared to affect the resistance to thermal inactivation afforded the enzyme. For example, in the temperature range 55–80°C where inactivation of the control was apparent, pressure of 100 MPa offered less protection to denaturation than did a pressure of 150 MPa.

In comparing the activity of enzymes at different pressures consideration should be given to the composition of the buffer. The assay system reported here utilizes phosphate buffer and a slight pH change (approximately 0.5 pH units) can be expected under pressure (Morild, 1981). The magnitude of the changes observed in Fig. 1 is unlikely to be due to pH variation. Blackwood and Mandl (1961) have shown that the hydrolysis of BANA by a crude cathepsin B preparation was increased by approximately 8% with a decrease in pH from 6.0 to 5.5. This is considerably less than the pressure-induced changes reported here.

The results of experiment 2 presented in Fig. 2 show the effect of three selected incubation temperatures on the activity of cathepsin B1 at 150 MPa. The maximum, minimum and optimum temperatures from Fig. 1 were examined to gain further insight into the interaction of temperature and pressure. Although the sample under 150 MPa pressure showed greater enzyme activity than the control at 80°C in Fig. 1, Fig. 2 shows that the enzyme reaction was complete at or before 10 min and the enzyme had been inactivated. Thus, it appears that 150 MPa pressure did not appear to protect the enzyme against thermal denaturation at 80°C. However, at 60°C, a temperature sufficient to inactivate the atmospheric pressure control in Fig. 1, the enzyme appeared very active, even after 60 min incubation. While there was no inactivation of the enzyme at 30°C, the rate of reaction at 60°C was higher than at 30°C and can be attributed to the effect of the higher incubation temperature.

The results presented here show that not only was the activity of cathepsin B1 retained under the elevated pressures used, but in some pressure-heat combinations it was greatly enhanced. For example, the activity of the enzyme at 60°C and 150 MPa was approximately 12 times that at 60°C and atmospheric pressure.

Although it is not possible to directly translate the results obtained with this assay system to the tenderization of meat during pressure-heat treatments, Bouton et al. (1977b) have reported maximum tenderizing of meat after pre-heating at 45°C for 45–180 min followed by pressure-heat treatment of 150 MPa at 60°C for 30 min. This combination of pressure-heat treatments corresponds to the conditions promoting maximum enzyme activity in the assay system reported in this study. It is possible that an increase in cathepsin B1 activity accounts,

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Detection of Enterotoxigenic *Escherichia coli* in Foods by DNA Colony Hybridization

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ABSTRACT

A DNA colony hybridization procedure was used to identify and enumerate a heat-labile toxin-producing strain of *Escherichia coli* (H10407) in various types of foods. Foods were seeded with H10407 cells and examined by DNA hybridization on nitrocellulose filters with ^{32}P-labeled heat-labile toxin gene fragments. The number of cells recovered on plate count agar and eosine methylene blue agar was compared. With nitrocellulose filters, recoveries were about 81% on plate count agar and about 76% on eosine methylene blue agar. No significant differences in recovery were observed with ten different food types.

INTRODUCTION

CERTAIN STRAINS of *Escherichia coli* carry the genes for enterotoxin production in plasmids (Gyles et al., 1977). Plasmids carry genetic information for other pathogenic determinants, such as host-specific colonization (Evans et al., 1975) and the ability to cause extra-intestinal (invasive) infections in a host (Williams et al., 1978). Laboratory procedures used to identify pathogenic traits include immunological, tissue culture, and animal tests (Dean et al., 1972; Donta et al., 1974; Sack and Sack, 1975; Yolken et al., 1977). However, the results obtained with these procedures are sometimes inconsistent, and the methods are not well suited for screening large numbers of cultures (Mehlman and Romero, 1982). Enterotoxigenic *E. coli* (ETEC) cultures that possess the genetic ability to produce heat-labile enterotoxin (LT) can be detected by DNA hybridization, using radioactively labeled LT gene fragments (Moseley et al., 1980). In this study, the DNA colony hybrizidation method was used to identify and enumerate an LT-producing ETEC (strain H10407) in a variety of food types.

MATERIALS & METHODS

Bacterial strains

ETEC strain H10407 was obtained from Dr. Joseph Lovett, Food and Drug Administration (FDA), Cincinnati, OH. This strain was previously examined using the Y-1 adrenal cell tissue culture test for LT and the suckling mouse procedure for detection of heat-stable toxin (Dean et al., 1972; Mehlman et al., 1978). The culture was incubated for 18 hr at 35°C in trypticase soy broth (TSB); cells were harvested at 5°C after centrifugation for 10 min at 6,000 × *g*, washed twice, and suspended in sterile normal saline. The saline cell suspension was held at 5°C before use. Appropriate dilutions of the saline cell suspensions were plated on plate count agar (PCA) medium to determine the number of colony forming units (CFU)/ml. Saline cell suspensions were discarded when the average cell counts decreased to log 0.3 of the original population.

Media

Growth media used for determining the most probable number (MPN) of *E. coli* and coliform cells as well as the total aerobic plate count (APC) were prepared according to instructions in the *Official Methods of Analysis* (AOAC, 1984). The PCA and Levine's eosine methylene blue (EMB) agar were each prepared according to the manufacturer's (Difco Laboratories, Detroit, MI) instructions.

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Table 1—Recovery of E. coli strain H10407 from seeded foods

Food	Recovery (%)[a]	
	PCA[b]	EMB[b]
Lettuce	94	107
Nonfat dry milk	65	70
Mixed fruit	107	82
Cream puffs	94	100
Breaded shrimp	57	53
Cream pie	86	79
Pecans	83	83
Deviled crabmeat	76	52
Frozen shrimp	62	37
Crabmeat	85	96
Average	81 ± 15.9	76 ± 22.8

[a] Recovery (%) = no. of dark spots observed on autoradiograms × 100/no. of cells seeded per filter.
[b] PCA, plate count agar; EMB, eosine methylene blue agar.

DNA colony hybridization

An 850 base-pair *Hin*dIII restriction endonuclease fragment of plasmid pEWD299 was used as a genetic probe for LT DNA (Dallas et al., 1979). This fragment contains the genetic information for all of the B subunit and a portion of the A subunit of the LT. Plasmid DNA isolation, restriction endonuclease fragment generation and purification, DNA labeling by nick translation (using ^{32}P-deoxycytidine triphosphate), and the colony hybridization procedure were based on the method of Moseley et al. (1980) as modified by Hill (1981) and Hill et al. (1983).

Analysis of foods by DNA colony hybridization

Ten samples, representing a wide range of foods, most of which are likely to be consumed without further cooking, were obtained from local retail sources: a dry fruit mixture (apples, apricots, and raisins), shelled pecans, nonfat dry milk (NFDM), cheese, frozen crabmeat, deviled crabmeat, breaded shrimp, cream puffs, refrigerated cream pie, and fresh lettuce. Except for the NFDM, all foods were homogenized with sterile Butterfield's phosphate buffer (AOAC, 1984) (1:9 w/v) for 2 min at high speed in a blender. The NFDM was rehydrated in sterile water according to the manufacturer's (Carnation Co., Los Angeles, CA) instructions and then homogenized. All foods were assayed for *E. coli* and coliform MPN and for APC/g of food by the recommended AOAC (1984) procedures.

Nitrocellulose filters (type BA-85, Schleicher and Schuell, Keene, NH) were boiled for 2 min in distilled water, placed between filter papers, wrapped in aluminum foil, and sterilized by autoclaving for 15 min at 121°C on the slow exhaust cycle. Before seeding with the test culture, all the food products were examined for LT-producing *E. coli*. The sterile filters were placed on the surface of PCA and EMB plates and then inoculated with 0.1 mL of a 1:10 dilution of food homogenate or rehydrated NFDM. A sterile bent glass rod was used to spread the inoculum evenly over the surface of the filter. The agar plates containing the filters were then incubated at 35°C for 24 hr; the resulting colonies were lysed and subjected to the DNA colony hybridization procedure. The 1:10 dilutions of food samples and NFDM were also seeded with appropriate phosphate buffer dilutions of the H10407 saline cell suspensions for subsequent analysis by DNA colony hybridization. A 1-mL volume of buffer containing 4×10^4 to

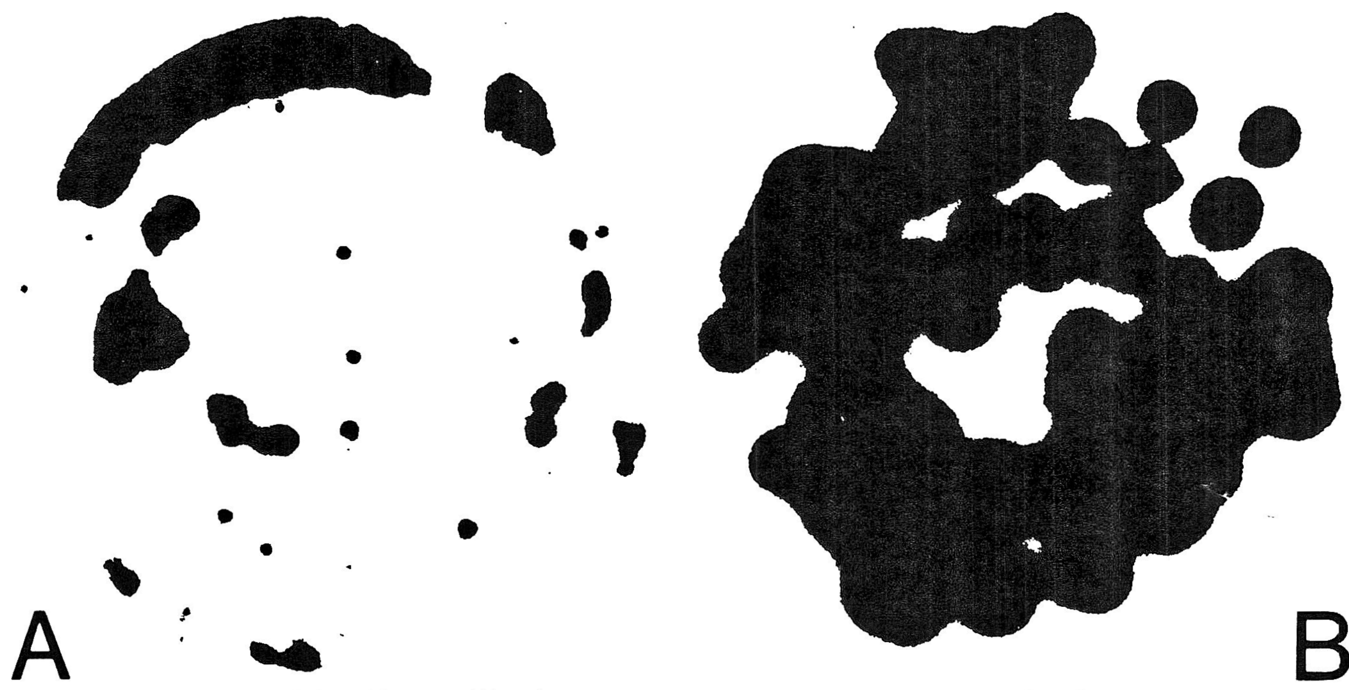

Fig. 1—Effect of eosine methylene blue agar (A) and plate count agar (B) on size of dark spots on autoradiograms.

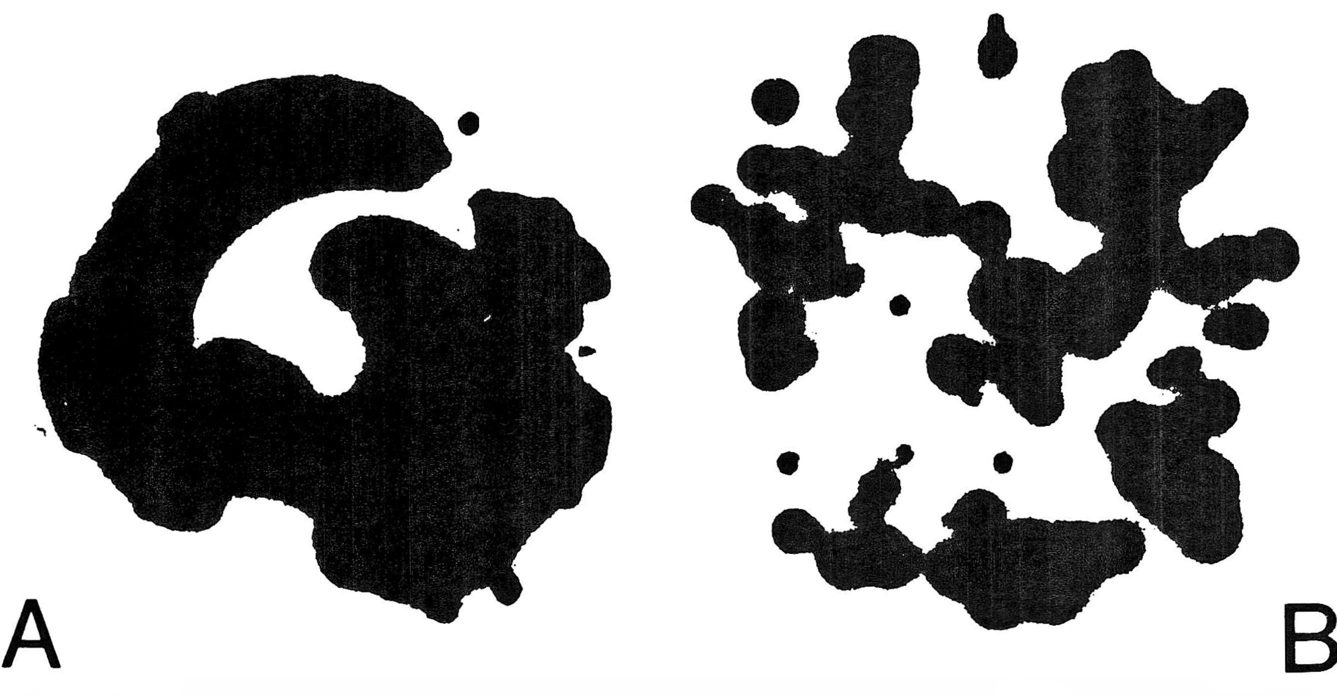

Fig. 2—Effect of ETEC H10407 spreading on autoradiograms. Wide, dark area on autoradiogram A was due to presence of food particles on nitrocellulose filter. Autoradiogram B shows result of large colonies close together, but no spreader type growth on nitrocellulose filter.

3×10^5 cells was mixed into 99 mL of each product, yielding 4×10^2 to 3×10^3 organisms per mL of product. A 0.1-mL portion of spiked product contained 50–300 organisms, which were spread-plated onto nitrocellulose filters.

After the colony hybridization process, the nitrocellulose filters were incubated with X-ray film at −20°C for 3 days. Each dark spot on the autoradiogram was considered to represent the recovery of a single H10407 cell. In some cases it was impossible to determine accurately the degree of recovery for the seeded H10407 cells because of the large colony size, which yielded even larger dark spots on the autoradiograms. However, the overlapping of the dark spots interfered with quantitative determinations only when colonies were not adequately separated from each other.

RESULTS & DISCUSSION

Recovery of H10407 from food samples

No *E. coli* cells were detected in any of the unspiked foods by the AOAC (1984) method. However, before spiking, one shrimp sample contained 43 coliforms (MPN/g), and both the shrimp and one crabmeat sample contained significant numbers of aerobic organisms (3.5×10^6 and 5.2×10^5/g, respectively) on PCA medium. Foods with unusually high aerobic plate counts also yielded the lowest percentage of recoveries of H10407 on PCA and EMB media. After spiking with H10407,

competition between this strain and the organisms already present in the foods gave lower recoveries than did food products with relatively low counts, such as NFDM and cream puffs. No LT-positive *E. coli* cells were detected in the nonseeded food samples by DNA colony hybridization. When the seeded samples were tested, an average of 81 and 76% recovery of the seeded H10407 cells was obtained with PCA and EMB, respectively (Table 1). Although the number of cells seeded onto PCA and EMB varied among food samples, the average recovery of H10407 on these two media (81 and 76%, respectively) was not significantly different (two-tailed t-test, $p = 0.58$). Colonies on PCA plates and the corresponding dark spots on the autoradiograms tended to be larger than on EMB plates (Fig. 1). During the analysis of crabmeat and frozen shrimp samples, spreading of the H10407 organisms on the filter interfered with the enumeration of dark spots on the autoradiogram particularly when large particles of food product were present (Fig. 2). Colonies tended to spread and coalesce around the edges of food particles on the filter. To eliminate this problem, the blended food homogenate was allowed to settle for 5 min before removal of the 0.1-mL aliquot for nitrocellulose filter inoculation. Despite the spreader-type growth, the DNA colony hybridization technique detected the LT-positive ETEC on the nitrocellulose filter. Other types of interference, such as inhibition of H10407 cell growth by a particular food, were not observed.

The DNA colony hybridization method is sensitive enough to detect as few as 100 cells/g of food when the food samples are diluted 1:10 in phosphate buffer; it has also been used to detect H10407 in scallops (Hill et al., 1983).

The recovery of H10407 on EMB was nearly equal to that on PCA by DNA colony hybridization. However, it would be better to use EMB when ETEC are present in foods highly contaminated with coliform microorganisms. The EMB agar would inhibit most non-coliforms from overgrowing ETEC, which may be present in lower numbers in some foods.

The sensitivity of the procedure could possibly be increased by a short incubation period (2-4 hr) in lauryl sulfate tryptose (LST) broth at a slightly elevated temperature (41.5°C) before removing 0.1 mL of inoculum for plating on a nitrocellulose filter. Under these conditions, cell numbers of strain H10407 may increase over 200-fold in 4 hr (Ferreira et al., unpublished data). Furthermore, LST could be substituted for phosphate buffer as a diluent for the food. This procedure was found to be well suited for the analysis of the ten different foods tested.

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Cellulolytic Enzymes and Antibacterial Activity of *Auricularia polytricha*

J. Y. LU and A. Y. TANG

ABSTRACT

Auricularia polytricha, an edible fungus, was grown in the laboratory and examined for composition and its ability to hydrolyze cellulose and hemicellulose. On a dry weight basis *A. polytricha* was highest in protein (7.59%), followed by fiber (3.69%) and lowest in fat (1.12%). Appreciable amounts of calcium and zinc were also present in the fruiting body of *A. polytricha*. The fungus produced substantial amounts of cellulase and hemicellulase. The optimum conditions were pH 4 and 30°C for cellulase, and pH 6 and 60°C for hemicellulase. *A. polytricha* also produced an antibacterial substance and inhibited the Gram positive bacteria, *Bacillus subtillus, Bacillus cereus, Micrococcus luteus*, and *Staphylococcus aureus*, but not Gram negative bacteria, *Alcaligenes viscolactis, Escherichia coli*, and *Pseudomonas fluorescens*.

INTRODUCTION

AURICULARIA POLYTRICHA, called "Mur-Erh" or "The Wood Ears", is an edible fungus that often grows on decaying trees in the forest. The fungus has been consumed as a common food ingredient in the Orient for many centuries. Geographical distribution, morphological characteristics, and cultivation techniques were described by Chang and Hayes (1978). Some interesting medicinal effects of *A. polytricha* such as hypolipidemic (Hammerschmidt, 1980), hypocholesterolemic (Kaneda and Tokuda, 1966) and inhibition of blood platelet aggregation (Hokama and Hokama, 1981; Makheija and Bailey, 1981) have been observed. Because of interest in growing this fungus, a number of studies have been done to grow it artificially on wood logs and sawdust for commercial production (Chang and Hayes, 1978; Chang and Tu, 1976; Wu, 1976).

Since *A. polytricha* can grow on wood, an attempt was made to investigate cellulolytic enzymes produced by this fungus. The composition of fruiting body and antibacterial activity was also investigated.

MATERIALS & METHODS

The culture

The fungus culture was provided by Mr. Y. M. Liao of the Agricultural Research Institute, Taichung, Taiwan. Difco's potato dextrose agar medium was used to grow and maintain the culture.

Proximate composition and minerals

Protein, fat, ash and moisture were determined according to AOAC (1980). The minerals, Ca, Fe and Zn, were determined by using a Perkin Elmer Model 3030 atomic absorption spectrometer (Anonymous, 1971a).

Solid medium

The medium which was used to produce fruiting body consisted of 88% sawdust (primarily of oak obtained from a local lumber mill), 10% wheat bran, 2% corn meal and 0.05% $CaCO_3$. These ingredients were mixed well, packed tightly in one-pint (470 mL capacity) Mason jar, moistened with adequate amounts of water, then sterilized at 120°C, 15 psi for 30 min. About 1 wk old *A. polytricha* culture grown on Difco's potato dextrose agar medium was employed as the inoculum. After inoculation, the jars were placed in a chamber where temperature was maintained at 28–30°C and the relative humidity at 80–90%. When the medium was covered fully with the fungus and the mycelia started to penetrate deep into the medium, the lid was opened, and the jars were watered two to three times daily with distilled water.

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Liquid medium

This medium was used to determine extracellular enzymes of *A. polytricha*. The fungus was grown in a 250 mL Erlenmyer flask containing 50 mL of the medium with the following composition: 1% glucose, 0.1% yest extract, 0.1% KH_2PO_4 and 5% sawdust. After inoculation, the fungus was grown stationary on the counter top in the laboratory for 2 wk at 25–30°C.

Enzyme assay

After incubation, the culture was centrifuged at 10,000 × *g* for 30 min to obtain a clear supernatant. The clear solution was used as the enzyme source.

Cellulase activity was determined by employing Whatman No. 1 filter paper as substrate as described by Mandels et al. (1974). Hemicellulase activity was determined by incubating 2 mL of substrate (3% xylan or locust bean gum in 0.1M citrate buffer, pH 6) with 1 mL of the enzyme solution for 2 hr. The substrates, highly purified xylan and locust bean gum, were purchased from Sigma Chemicals (St. Louis, MO). The reducing sugars liberated were determined by the dinitrosalicylic acid method (Miller, 1959). The protein content of the enzyme solution was determined by Lowry's method (Lowry et al., 1951).

Antibacterial activity

The organisms tested, *Bacillus subtillus, Bacillus cereus, Micrococcus luteus, Staphylococcus aureus, Alcaligenes viscolactis, Escherichia coli*, and *Pseudomonas fluorescens* were provided by the Dept. of Microbiology, School of Veterinary Medicine, Tuskegee University. The bioassay method described by Wong and Bau (1977) was employed to examine antibacterial activity. The petri dishes were incubated at room temperatures (20–25°C) and a clear zone of inhibition surrounding the fungal colony measured.

RESULTS & DISCUSSION

AFTER 4 WK INCUBATION, fruiting bodies were harvested. They appeared leathery, elastic and gelatinous; cup or ear-shaped; one or several lobes, and had a red-brown color initially but turned dark with age. The largest size attained was about 10 cm in diameter. The composition of the fruiting bodies is shown in Table 1. Fresh *A. polytricha* fruiting bodies had high moisture, 91.8%. On a dry weight basis it had protein, 7.59%; ash, 1.45% and fat, 1.1%. These values agreed with those of Chang and Hayes (1978) except ash was lower. Differences in species and cultivation conditions and age of fungus may result in a wide variation in the composition of mushrooms. Mineral analysis indicated that *A. polytricha* had relatively high levels of iron (0,74 mg/g) and calcium (3.5 mg/g). The high calcium level observed in this study could be the result of adding $CaCO_3$ in the solid medium. The fruiting bodies of *A. polytricha* also contained an appreciable amount of zinc (0.04 mg/g) which compares favorably with some vegetables such as lettuce, peas, and brussels sprouts.

A substantial amount of cellulolytic activity was produced by *A. polytricha* (Fig. 1a and b). Since *A. polytricha* can be

Table 1—Proximate Composition*

Species	Moisture %	Protein %	Ash %	Fat %	Fiber mg/g	Iron mg/g	Calcium mg/g	Zinc mg/g
A. polytricha	91.8	7.59	1.45	1.12	3.69	0.74	3.51	0.04
*A.polytricha***	88.71	7.9	6.7	1.2	9.1	0.47	0.24	na***
A. polytricha# dried	13.1	5.5	0.4	1.0	4.6	0.16	0.27	na

* Data (protein, ash, fat and fiber) presented are on percentage dry weight basis
** Chang et al. (1978)
*** na — not available
# Anonymous (1971b)

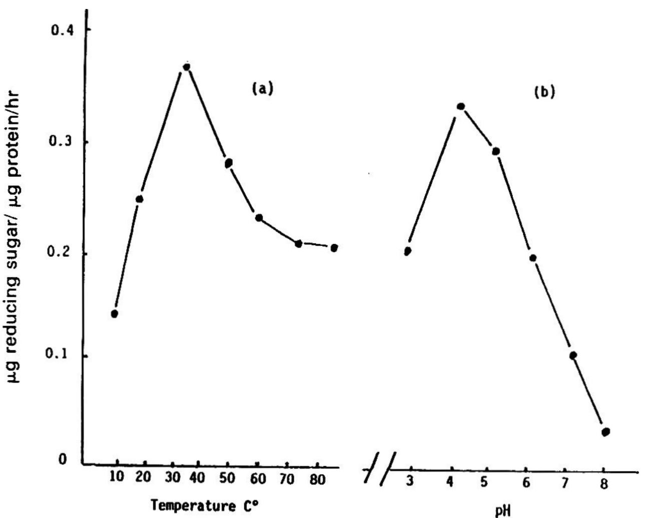

Fig. 1—Effect of temperature and pH on cellulase activity.

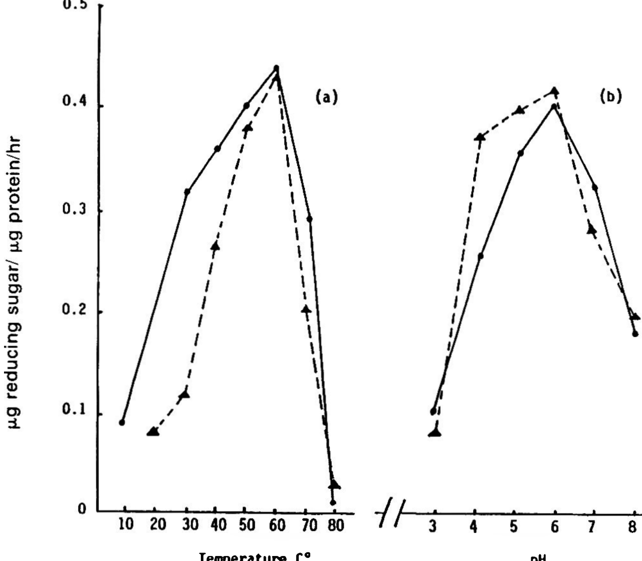

Fig. 2—Effect of temperature and pH on hemicellulase activity. ● —— ● xylan; ▲ - - - - ▲ locust bean gum.

grown on wood, cellulase production was expected. The highest activity was 0.37 µg glucose/µg protein/hr at 30°C. It is interesting to know that the optimum temperature was 30°C, considerably lower than other fungal cellulases such as *Coniophora cerebella*, 50°C (King, 1966), *Thermoascus*, 60°C (Tong et al., 1980) and *Trichoderma viride* 50°C (Mandels et al., 1974). *A. polytricha* cellulase was thermoduric. An appreciable amount of activity still remained after incubation for 1 hr at 70°C. The optimum pH of *A. polytricha* cellulase was 4 which appeared slightly lower than other cellulolytic fungi such as *Sclerotium rolfsii*, pH 4.5 (Shewale and Sadana, 1979) and *Trichoderma viride*, pH 5.2 (Mandels et al., 1974).

A. polytricha also produced hemicellulase. The effect of temperature and pH on the enzyme activity is shown in Fig. 2a and b. The highest activity observed was 0.41 µg reducing sugar/µg protein/hr at 60°C. In this study, although two substrates (xylan and locust bean gum) were employed, the optimum conditions appeared to be the same, 60°C and pH 6.0. Xylan consists mainly of xylose while locust bean gum contains a high proportion of mannose. The result indicated that *A. polytricha* hemicellulase included two enzymes; xylanase and mannanase which were equally active. Generally, fungal hemicellulase is most active in a temperature range of 37–60°C (Wankhede et al., 1981). The optimum pH of *A. polytricha* hemicellulase was 6.0. This value appeared higher than for other fungi, such as *Trichoderma roseum*, pH 4.6, *Aspergillus niger*, pH 4.6 (Salmanova and Zhanova, 1975) and *Aspergillus terreus*, pH 5-6 (Loginova and Tashpulatov, 1978). One possible application of hemicellulase may be to degrade gum in biomass and solve the problems of pumping and filtering.

The present study indicated that *A. polytricha* produced relatively active cellulase and hemicellulase. These enzymes could be used to convert biomass to fermentable sugars for subsequent fermentation such as ethanol or single cell protein production.

Some fungi imperfecti such as *Penicillium* are known to produce antibiotics, but antibacterial activity of higher fungi is not well documented. In this study, *A. polytricha* was tested against several bacteria. The results showed that the diameter of inhibition zone (in mm, mean ± S.D. of two determinations) of Gram positive bacteria was 3.75 ± 0.35 for *B. subtilus*; 3.70 ± 0.28 for *B. cereus*; 2.00 ± 0.28 for *M. luteus*; and 2.50 ± for *S. aureus*, but no inhibitory zone was observed with Gram negative bacteria, *A. viscolactis*, *E. coli* 0138, *E. coli* 0148 B, and *P. fluorescens*. It is interesting to note that *A. polytricha* was effective against *S. aureus*, one of the common foodborne disease bacteria that often causes public health problems. It is not known at this time what is the nature of this effective substance produced by *A. polytricha*. The substance could be an antibiotic, a lytic enzyme or an antinutritive factor. Further study is needed to isolate, identify and investigate the potential use of this fungal metabolite as related to food and public health.

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Stability Comparison of Two Betacyanine Pigments — Amaranthine and Betanine

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ABSTRACT

The stability of amaranthine in solution was compared to the stability of betanine in solution. Amaranthine was extracted from fresh leaves (*Amaranthus tricolor*). HPLC was used for the quantification of amaranthine, betanine and their degradation products. The degradation reaction of amaranthine, as with betanine, is a partially reversible reaction. The equilibrium constant at 90°C for amaranthine degradation was determined to be 0.125 mM compared to 0.071 mM for betanine. Amaranthine is less stable than betanine in the absence of oxygen, while in the presence of an excess of oxygen, the stability of the two pigments is essentially equal.

INTRODUCTION

AMARANTH (*Amaranthus tricolor*) is a widely distributed but underutilized vegetable (Herklots, 1972; Teutonico and Knorr, 1985). More than fifty species of amaranth are known to exist. It is consumed either fresh as "greens" or at the mature state as grain. The red leafed vegetable is harvested for fresh consumption 28–30 days after sowing, when the plant has reached a height of 12–15 cm (Herklots, 1972). The seed is harvested at maturity, 90–120 days after planting (Edwards, 1980). The red color appears in the leaf and stem and the intensity varies among species. The red pigment responsible for the color was found to be a betacyanine (Mabry and Dreiding, 1968) and was named amaranthine after its source. It was identified as the 5-0-[2-0-(β-D-glycopyranosyluronic acid)-β-D-glucopyranoside] (Fig. 1) of betanidine (Piatelli et al., 1964, Piatelli and Minale, 1966).

Betanine has been used as a colorant in many foods (von Elbe, 1977). Factors affecting its stability during processing such as pH value, temperature, oxygen, light and water activity have been extensively studied (von Elbe et al., 1974; Sapers and Hornstein, 1979, Pasch and von Elbe, 1979; Saguy, 1979; Attoe and von Elbe, 1982). The reported data show that betanine is heat labile, and the thermal stability is pH and oxygen dependent. Removal of oxygen greatly increases its stability toward heat. The degradation products of betanine in solution upon heating have been identified as betalamic acid and cyclodopa 5-0-glucoside (Schwartz and von Elbe, 1983). Exposure of betanine in solution to light at low temperatures (20°C) significantly affected its stability, but at elevated temperatures (40°C) the degradation due to heat was such that light exposure became insignificant (Attoe and von Elbe, 1981). The degradation reaction of betanine in solution is partially reversible (von Elbe et al., 1981; Huang and von Elbe, 1985). This regeneration of the pigment must be considered when determining total betanine losses under different experimental or processing conditions.

A red colored extract of amaranth leaves has been used to color foods in different countries throughout the world (Teutonico and Knorr, 1985). However, no data exist on the effect of processing parameters on its stability. Since amaranthine is easily obtainable and may have a potential use as a colorant

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BETANIDINE
R = H

BETANINE
R = β-D-glucosyl

AMARANTHINE
R = 2'-O-(β-D-glucosyluronic acid)-β-D-glucosyl

ISOMER IS THE C-15 EPIMER

Fig. 1 — Structure of betanidine, betanine and amaranthine.

the purpose of this study was to: (1) evaluate the effect of heat, pH value, oxygen and light on the stability of amaranthine in solution, and (2) make stability comparison between amaranthine and betanine.

MATERIAL & METHODS

Preparation of crude plant extract

Amaranth was grown on the experimental farm of the University of Wisconsin-Madison. Fifty days after planting the fresh leaves were harvested and stored immediately at − 15°C for no more than 2 days. The leaves were blanched at 80°C for 5 min. The blanch water, which contained large amounts of pigment, was cooled over a surface heat exchanger and saved. The blanched leaves were blended twice with water in a 4L Waring Blendor. The mixture was filtered through multilayers of cheesecloth and the filtrate was combined with the blanch water and frozen at − 15°C until further used.

Purification of amaranthine

Amaranthine was purified following essentially the method of Schwartz and von Elbe (1980) for the purification of betanine. The differences were pH adjustment of the pigment extract and changes in chromotographic conditions. The aqueous pigment extract was adjusted to a pH value of 2.7 with concentrated HCl. Any precipitate that formed upon acidification was removed by centrifugation. Five hundred milliliters of extract was placed on a Sephadex G-25 column (Pharmacia K 100/100) previously washed with 1% acetic acid. The column was eluted with deionized water. The major red pigment band (approximately 1L) was collected and freeze dried. Further purification was accomplished by preparatory HPLC. Ten milliliter samples were chromatographed on four 7.8 mm ID × 61 cm Bondapak C_{18}/Porasil B columns connected in series (Waters Assoc., Milford, MA) with a mobile phase consisting of CH_3CN/0.005M Pic A (Waters Assoc., Milford, MA) (10:90 v/v). Two fractions of the red pigment, one minor and one major, were separated. The major fraction was rechromatographed using a mobile phase of CH_3OH/0.005M KH_2PO_4 (12.5/87.5 v/v). This step in the purification was done to assure separation of betalamic acid from amaranthine. The fraction was freeze-dried and stored at − 15°C until further use.

The pigment was dissolved in 0.1% HCl and held at −20°C to initiate crystallization. The crystals were collected by centrifugation and dried under vacuum. The 1% absorptivity value in water of the pigment was determined as 786. This value compared well with the reported literature value for amaranthine of 779 (Piatelli et al., 1969).

The purified pigment was subjected to enzymatic degradation by

Table 1—Storage stability of amaranthine in solution at different pH values, stored at 4°C for 7 days

pH value A_{max} (±0.05)	Storage time 0 days A^a (±0.01)	$A_{max}{}^b$ ± (1 nm)	7 days A (±0.01)	± (1 nm)	Pigment retention (%) Amr[c]	Bet[d]
2.0	0.39	535	0.27	534	69	72
3.0	0.48	536	0.41	535	85	92
4.0	0.50	537	0.46	536	92	98
5.0	0.50	538	0.48	538	96	98
6.0	0.50	538	0.49	538	98	96
7.0	0.49	538	0.46	538	93	90
8.0	0.47	540	0.36	540	77	76
9.0	0.43	542	0.30	542	79	69

[a] A = light absorption
[b] A_{max} = maximum wavelength
[c] Amr = amaranthine, Bet = betanine
[d] from von Elbe et al., 1974

Table 2—Thermal stability of amaranthine in solution at pH 5.0 compared to betanine

Reaction Condition Pigment conc(mM)	Temp (°C)	$O^a{}_2$	Reg[b]	1st $t_{1/2}$ (min)[d] Amr[c]	Bet	2nd $t_{1/2}$ (min) Amr	Bet
0.25	65	−	−	1104±65	1746±90	2016±120	2874±162
0.25	65	−	+	1476±65	2088±50	2010±175	2546±114
0.027	65	+	−	88±4	80±3	86±5	81±3
0.027	65	+	−	94±8	83±7	90±10	80±10
0.25	90	−	−	19±2	44±3	99±9	144±6
0.25	90	−	+	88±5	112±7	98±9	108±12
0.027	90	+	−	12.8±1.2	12.8±1	125±1.3	128±1.5
0.027	90	+	+	14.4±2.1	15.6±25	17.6±2.2	19.0±3.0

[a] Oxygen absent (−); oxygen present (+); before regeneration (−); after regeneration (+)
[b] Amr = amaranthine, Bet = betanine
[c] Regeneration excluded (−); regeneration included (+)
[d] $t_{1/2}$ = half - life value

Table 3—Comparison of rate and equilibrium constants for the degradation reaction of amaranthine and betanine

Rate	Constant	Temperature (°C) 90	75	65	Ea (Kcal/mole)
$k_1{}^a$	Amaranthine	0.058 ±0.004	0.018 ±0.001	0.0086 ±0.005	17.9 ± 0.9
	Betanine[b]	0.061 ±0.003	0.020 ±0.001	0.0101 ±0.0005	17.3 ± 0.8
k_{-1}	Amaranthine	0.467	0.430	0.408	1.32
	Betanine	0.867	0.833	0.810	0.64
Equilibrium Constant					ΔH (KJ/mole) Kcal/mole
K	Amaranthine	0.125 ±0.005	0.042 ±0.003	0.0211 ±0.0008	6.9 16.4
	Betanine	0.073 ±0.003	0.025 ±0.001	0.0125 ±0.0005	7.1 16.9

[a] k_1 - first order rate constant (min^{-1}); k_{-1} = second order rate constant min^{-1} mM^{-1}
[b] from Huang and von Elbe, 1985.

β-glucuronidase or β-glucosidase to form betanine or betanidine, respectively. The later products were compared to betanine and betanidine derived from red beets by the method of Schwartz and von Elbe (1980).

Sample preparation and reaction conditions

Samples were prepared by dissolving appropriate amounts of pigment in a 0.1M citric-phosphate buffer at a specific pH value. Buffer solutions with pH values of 2.0 and 9.0 were prepared by the addition of 1N HCl or 1N NaOH, respectively. All samples were filtered through a 0.45m HA Millipore filter, (Millipore Corp., Bedford, MA). Two milliliter glass vials fitted with sleeve-type rubber stoppers were used as reaction vessels. Oxygen-free solutions were obtained by flushing nitrogen through the pigment solutions for 10 min. Ninety-five to ninety-nine percent of the dissolved oxygen was removed within 2 min (Attoe and von Elbe, 1982). The samples were held at the desired temperature in a controlled water bath for various time intervals. For each time and temperature condition, six vials were removed from the water bath. Triplicate analyses for pigment concentration were conducted immediately after the reaction time and after holding the sample at room temperature for 2–20 hr. Various holding times were used to allow for maximum regeneration of amaranthine or betanine at each condition. Vials were wrapped in aluminum foil to prevent exposure to light during the heating periods (Attoe and von Elbe, 1981). Samples were exposed to 210 ft-c during the heating times if the light effect was to be determined. A 2^3 factorial experimental design (Table 2) was used to compare the three conditions on the stability of amaranthine and betanine. The concentrations for amaranthine and betanine were maintained at equal molar concentration for each condition. The pigment concentration was 0.25 mM when oxygen was absent. The residual oxygen after nitrogen flushing was 0.01 mM (Attoe and von Elbe, 1982), and therefore a pigment excess was assured. The pigment concentration was 0.027 mM when oxygen was present. This pigment concentration was chosen to assure an excess oxygen level. The con-

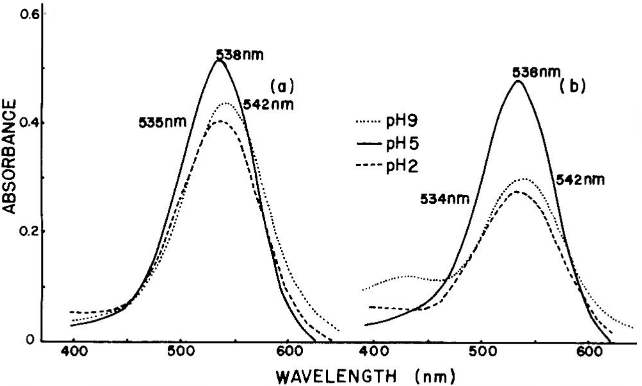

Fig. 2 — Absorption spectra in the visible region of amaranthine in solution at pH 2.0, 5.0 and 9.0. (a) before storage; (b) after storage of 7 days at 4°C.

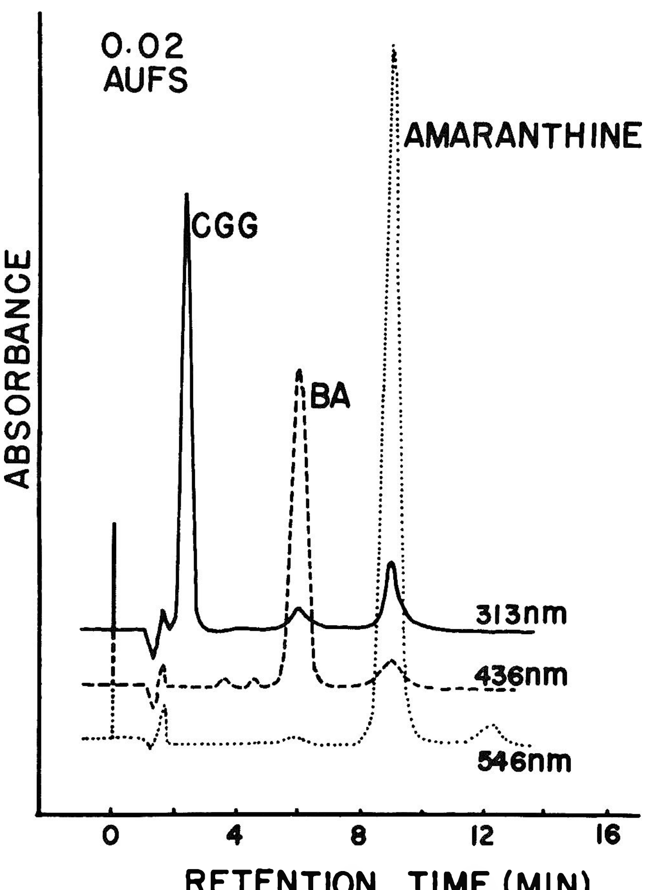

Fig. 3 — HPLC chromatograms of amaranthine in solution under nitrogen after heating at 90°C for 30 min monitored at 546, 436 and 313 nm. Betalamic acid = BA; Cyclodopa-5-0-[2-0-(β-D-glucuronic acid)-β-D-glucoside] = CGG.

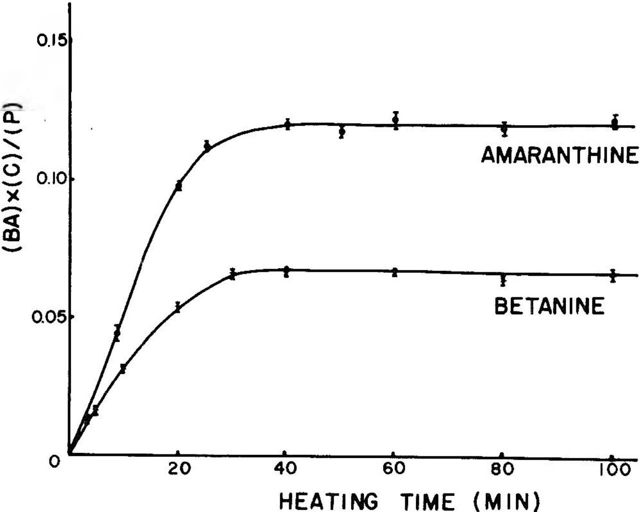

Fig. 4 — Ratio of BA × C/P vs. reaction time at 90°C under nitrogen. BA = betalamic acid; C = Cyclodopa-5-0 glucoside or Cyclodopa-5-0-(2-0-glucuronic acid) glucoside; P = betanine or amaranthine.

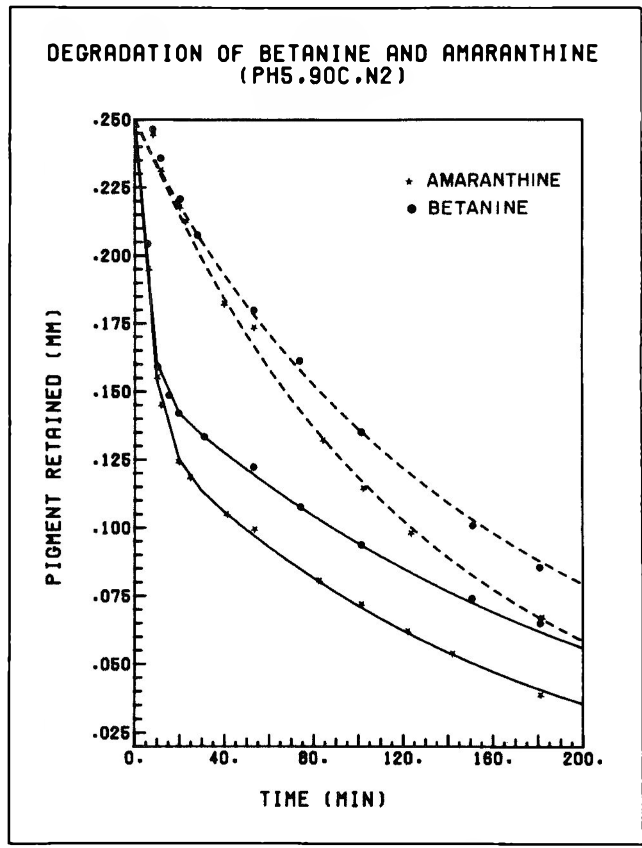

Fig. 5 — A computer simulation of amaranthine and betanine degradation before regeneration (solid lines); after regeneration (dashed lines).

centration of oxygen in water at the temperature of the study was 0.14 and 0.09 mM at 65 and 90°C, respectively (Camp, 1963).

Half-life values, instead of rate constants, were used as index of stability for each pigment. This parameter was chosen because the reaction order kinetics for the degradation reaction differ depending upon the absence or presence of oxygen (Attoe and von Elbe, 1982). In the calculation of the half-life values the concentrations of isobetanine and isoamaranthine were calculated as betanine and amaranthine, respectively.

Sample analysis

Visible spectra of amaranthine in solution at various pH values were measured using a Beckman Model-25 spectrophotometer with specific buffer solutions as the reference. The concentration of amaranthine or betanine in solution was determined by HPLC. Columns (μ Bondapak C_{18}) were developed isocratically with 8% CH_3CN in 5 mM Pic A solution as the mobile phase for amaranthine and its degradation products, and 12% CH_3CN in 5 mM Pic A solution for betanine and its degradation products. The chromatograms were monitered at 546, 436 and 313 nm.

Data analysis

Replicate determinations were averaged to obtain a single datum point for each reaction condition. Nonlinear regression analysis and non-linear differential equation, which are required for the calculation

Table 4—Effect of pigment concentration and oxygen levels on the light influence (210 ft-c) at 25°C stored for 50 hr

Sample		% Amaranthine retained ± SD	
Amaranthine Conc. (mM)	Oxygen[a]	no light exposure	light exposure
0.03	+	68 ± 3	47 ± 4
0.03	−	84 ± 5	83 ± 4
0.25	+	78 ± 4	54 ± 4
0.25	−	92 ± 2	90 ± 3

[a] Oxygen absent (−); oxygen present (+)

of rate constants and equilibrium constants, were solved by a Sperry Univac 1100 computer system (Academic Computer Center, University of Wisconsin-Madison).

RESULTS & DISCUSSION

pH effect

The effect of pH on amaranthine in solution between 2.0 and 9.0 was determined initially and after 7 days of storage at 4°C. The changes in the visible spectrum for solutions at pH 2.0, 5.0 and 9.0 are illustrated in Fig. 2a and b. Light absorbence value (A), absorption maxima (A_{max}) and the % pigment retention are presented in Table 1. Percent pigment retention was calculated in terms of

$$A_2/A_1 \times 100$$

where A_1 is the initial light absorption value and A_2 is the light absorption value after 7 days of storage at 4°C. Values for betanine retention were based on previously reported data by von Elbe et al. (1974). The changes in the visible spectra for amaranthine in solution as affected by pH were similar to those reported for betanine in solution. In the pH range 3.0–7.0 (the pH range of most foods) the spectra were unaffected by a change in pH value. Outside the range the light absorption of amaranthine in solution, as with betanine, shifted to a longer wavelength with a marked decrease in the A_{max} and an increase in the light absorption in the 575–650 nm region (Fig. 2a).

The stability of amaranthine solutions stored at 4°C for 7 days was similar to that of betanine in solution, with greatest stability in the pH range 5.0–6.0 (Table 1). At pH 5.0 there was little or no change in the visible spectrum and retention of the pigment was 96%. The spectrum of amaranthine (Fig. 2b) in solution at a pH value of 9.0 after storage showed a distinct peak at 430 nm. This light absorption is believed to be due to the formation of betalamic acid (Kimler, 1972). Isolation of the compound responsible for the peak by preparatory HPLC resulted in a yellow fraction (A_{max} 430), when co-chromatographed with betalamic acid obtained from betanine (Schwartz and von Elbe, 1983) gave a single peak. This is further evidence for the formation of betalamic acid when betacyanine solutions were subjected to high pH values.

Thermal-stability

The degradation reaction of betanine in solution at pH 5.0 was greatly influenced by the temperature of the reaction, the presence or absence of oxygen and whether or not the experimental conditions involved allowed for the regeneration of betanine (Huang and von Elbe, 1985). The results of the factorial design experiment are listed in Table 2. The half-life values of amaranthine in solution were affected by the presence of oxygen and, as with betanine, were greater in the absence of oxygen. Regeneration, observed with betanine in solution, also occurred with amaranthine. In the presence of oxygen the half-life values for the pigments were virtually equal, but in the absence of oxygen, whether or not regeneration was complete, the half-life values for amaranthine were shorter than those for betanine. The oxygen effect on the half-life values was less pronounced at 90°C than at 65°C. The second half-life value for either pigment was longer in the absence of oxygen than the first half-life value. This increase in the half-life values confirms the deviation from first order reaction kinetics.

Determination of rate and equilibrium constants

Huang and von Elbe (1985) adapted a reaction model for betanine degradation at pH 5.0, which accounted for the reversibility of the reaction. Since the effects of pH, temperature and oxygen on the thermostability for amaranthine are similar or greater than for betanine in solution, it is feasible to apply the model to the degradation of amaranthine. Betanine in solution is known to degrade to betalamic acid (BA) and cyclodopa-5-0-glucoside (CDG) (Schwartz and von Elbe, 1983). The regeneration process involves a Schiff base condensation of the nucleophilic amine of CDG with the aldehyde of BA. This reaction most likely also exists when amaranthine is in solution because of the similarity in structure of the two betacyanine pigments. The degradation reaction can be written as follows:

$$\text{Amaranthine} \rightleftharpoons \text{BA} + \text{CGG} + H^+$$

CGG = Cyclodopa 5-0-[2-0(β-D-glucuronic acid)-β-D-glucoside]

Fig. 3 presents HPLC chromatograms of amaranthine in solution under nitrogen after heating at 90°C for 30 min. Monitoring at 546 nm detected amaranthine only, while monitoring at 313 and 436 nm detected the formation of CGG and BA respectively. The formation of BA was further shown by the addition of BA, derived from betanine, to a degradation mixture of amaranthine. The chromatogram showed an increase in the betalamic acid peak after the addition. The formation of CGG was further shown by the addition of β-glucuronidase to amaranthine to form betanine which upon heating resulted in the formation of CDG.

The concentration ratio of BA × CGG/amaranthine or BA × CDG/betanine, when plotted against the reaction time of amaranthine or betanine, at 90°C under nitrogen, reached a plateau (Fig. 4). The constant ratio obtained after approximately 30 min of reaction time was used to estimate the equilibrium constant (K) for amaranthine or betanine. The method for the calculation of the rate constants has previously been described by Huang and von Elbe, 1985. Values for k_1, k_{-1}, K, E_a, and $\triangle H$ for amaranthine in solution are listed in Table 3. The data were compared to previously reported data for betanine in solution. The values for k_1 for both pigments were similar. The K for amaranthine in solution was approximately twice as great as that for betanine and therefore the reverse rate constant k_{-1} for amaranthine was one-half as great as that for betanine. The greater K for amaranthine explained the greater heat sensitivity of the pigment in solution. The greater K favored the accumulation of BA and CGG, while a small K_{-1} value hindered the condensation of BA and CGG to amaranthine. This condition favored further degradation of BA.

A computer simulation of the degradation for amaranthine and betanine under nitrogen at 90°C using the calculated rate constants is illustrated in Fig. 5. Both pigments degraded following the same rate change, with the initial rate being faster than the later rate. The change in rates occurred after 20-30% of the pigments were degraded. However, because of the difference between the rate constants for the reverse reaction, less amaranthine was regenerated resulting in an overall lower stability (shorter half-life values) for amaranthine.

The differences in the stability toward heat between amaranthine and betanine can be explained when constructing a three dimensional model of the amaranthine molecule. Such a model revealed that the carbonyl group of the glucuronic acid portion of the sugar moiety is adjacent to the quarternary amine. The pK value of glucuronic acid according to Marsh (1966) is 3.20. The quarternary amine of amaranthine at pH 5.0, in contrast to that of betanine, is stabilized not only by the carboxyl group of the indole ring but also by the carboxyl group of glucuronic acid. The more stabilized quarternary amine of

amaranthine makes the condensation of BA and CGG more difficult because the nitrogen electrons are needed for the initiation of the condensation (Schiff base).

In the presence of oxygen the degradation of both pigments was essentially equal (Table 2), and approached first order reaction kinetics. The great sensitivity of BA to oxygen was the reason for the reaction to approach first order reaction kinetics under oxygen. Under this condition the reaction never reached equilibrium because of the small value of BA × C/P. The reaction rate, therefore, was controlled by k_1 which was equal for both pigments when oxygen was present (Table 3).

Effect of light

In the presence of an excess of oxygen, light reduced the stability of amaranthine in solution at low temperatures. The half-life at 25°C of a 0.03 mM solution of amaranthine not exposed or exposed to 210 ft-c intensity of light was 78 ± 8 hr and 46 ± 6 hr., respectively. At temperatures above 40°C the added influence of light on the degradation rate was undetectable. Similar effects of light were observed on the degradation rate of betanine in solution (Attoe and von Elbe, 1981). These authors noted that when pigment concentration was increased, combined with the removal of oxygen, the effect of light exposure on the degradation of betanine could be eliminated.

In this study a 2 × 2 factorial design (Table 4) was employed to study the effect of light on solution containing different concentration of pigment in the presence or absence of oxygen. The samples at pH 5.0 were maintained at 25°C for 50 hr. The results showed that an increase in pigment concentration alone had no effect on the overall stability of amaranthine in solution but that if oxygen was removed the light effect was negligible.

CONCLUSIONS

THE EFFECT of pH on the color and stability of amaranthine in solution was typical of betacyanines. In the pH range of most foods (3.0–7.0) the color of the pigment solution was unchanged, however, the heat stability changed and the pigment was most stable in the pH range 5.0–6.0. The thermal stability of amaranthine, as with betanine, was affected by the presence of oxygen. Amaranthine was less stable than betanine in the absence of oxygen, while in the presence of an excess of oxygen, the stability of the two pigments was equal. The equilibrium constant at 90°C for amaranthine degradation was 0.125 mM compared to 0.071 mM for betanine. Amaranthine degraded upon heating to BA and CGG. The degradation reaction, as with betanine, was partially reversible and involved a Schiff base. The stability of betacyanines in solution during heating, therefore, depended upon the imino structure (Schiff base). In the amaranthine molecule the quartenary amine is stabilized by the carboxylic group on both the indole ring and the glucuronic acid unit, which hindered the condensation of BA with CGG.

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1,6-Anhydro-3,4-O-Furfurylidene-β-D-Galactopyranose, A New Furfurylidene Derivative Formed from Lactose During Pyrolysis

TADASU URASHIMA, KYOZO SUYAMA, and SUSUMU ADACHI

ABSTRACT

A new fufurylidene derivative which tastes bitter was separated from lactose heated at 200°C for 20 hr by ethyl acetate extraction and preparative thin-layer chromatography. The compound was identified as the trans isomer of 1,6-anhydro-3,4-O-furfurylidene-β-D-galactopyranose by ultraviolet, infrared, mass and ¹H-NMR spectroscopy. The compound and its cis isomer were identified in the reaction mixture of furfural and levogalactosan heated at 175°C for 2 hr with nitric acid.

INTRODUCTION

MILK on heating gradually becomes brown. It is known that the Maillard reaction between lactose and amino compounds involving milk proteins and urea is the most prevalent in the color change, since it requires a relatively low energy of activation and is autocatalytic (Patton, 1955). In spite of this fact, caramelization of lactose alone would also have some role in browning and flavor formation of low moisture dairy products on heating at high temperature.

Lactose pyrolysis has been investigated as a part of nonenzymatic browning during thermal processing of foods containing lactose (Whittier, 1925-1926; Whittier, 1944; Nickerson, 1974; Hohno and Adachi, 1982; Hohno et al., 1983; Urashima et al., 1983, 1985). In the first step of pyrolysis, lactose hydrate lost all its water of crystallization followed by browning, with perceptible change in weight. From the pyrolysate many anhydro sugars, oligosaccharides and other substances have been detected as intermediate products in the course of pyrolysis of lactose (Hohno and Adachi, 1982; Hohno et al., 1983; Urashima et al., 1983).

In a recent study (Urashima et al., 1985), a new cyclic acetal condensed from levogalactosan with 5-(hydroxymethyl)-2-furaldehyde; 1,6-anhydro-3,4-O-[5-(hydroxymethyl)-2-furfurylidene]-β-D-galactopyranose, was separated from lactose pyrolysate. It was deduced that in the second step of pyrolysis some carbonyl compounds formed from lactose might be condensed with the sugars. Therefore, we tried to isolate the other cyclic acetal in the pyrolysates of lactose.

MATERIALS & METHODS

LACTOSE MONOHYDRATE and furfural were purchased from Wako Co. Anhydrous galactose was purchased from Tokyo Kasei Co. Other chemicals were commercial products of reagent grade. Levogalactosan was prepared from phenyl-β-galactoside by heating in 1.3N NaOH solution according to Montogomery et al. (1943). 1,6-Anhydro-3,4-O-isopropylidene-β-D-galactopyranose was prepared from levogalactosan by the usual way (Schmidt, 1963).

Melting points were measured on a micro melting point apparatus (Yanagimoto, Japan) and were not corrected. Optical rotations were measured on a JASCO DIP-4 digital polarimeter. UV and visible spectra were recorded on a JASCO UVIDEC-505X spectrometer. Infra red spectra were determined on a Hitachi 260-10 spectrometer from

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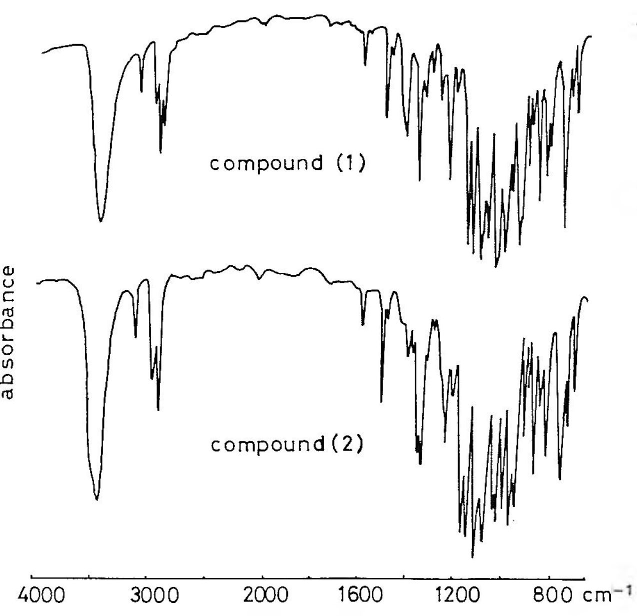

Fig. 1—Infrared spectra of compound (1) and compound (2) measured from KBr disks.

KBr disks. ¹H-NMR spectra were recorded at 100 MHz, with TMS as an internal standard on a JEOL-FX-100 spectrometer, operated in the pulsed Fourier transform-mode. ¹³C-NMR spectra were obtained with a JEOL-FX-100 spectrometer, operated at 25 MHz. Mass spectra were measured on a Hitachi M-52G mass spectrometer; ionization current and voltage were 100 μA and 25 eV, respectively. Field desorption MS was measured on a JEOL-JMS-OISG-2 mass spectrometer, accelerating voltage was 8 KV and cathode voltage was −5 KV. High resolution MS was performed with a JEOL-JMS-OISG-2 mass spectrometer; ionization current and voltage were 100 μA and 25 eV, respectively.

Gas-liquid chromatography (GC)

A Hitachi model 163 gas chromatograph equipped with a flame ionization detector and a steel column (0.2 × 100 cm) packed with a 10% SE-30 on chromosolv W was operated with a temperature gradient of 5°C/min from 100°C to 300°C.

Thin-layer chromatography (TLC)

Preparative TLC was performed with silicagel 60 or silicagel 60G and 9:1 dichloromethane — acetic acid (solvent A), 2:3 dichloromethane-ethyl acetate (solvent B), dichloromethane (solvent C) and 20:3 dichloromethane-acetone (solvent D) as developing solvents. 2,4-Dinitrophenylhydrazine (2,4-DNPhydrazine) 1%, in 3% HCl-MeOH solution was the spray reagent. Analytical TLC was run on silicagel 60, employing solvent C, solvent D or 10:5:4 butanol-isopropanol-water (solvent E) as solvent systems. The samples were detected by spraying with 5% H_2SO_4-MeOH and heating at 105°C for 10 min.

Isolation of the compound (1) from lactose pyrolysate

Lactose monohydrate (1800g), in a round bottom flask attached to a reflux condenser, was heated at 200°C for 20 hr. After heating for 8 hr, the temperature of the reactor was maintained at 120°C, while the sample melted and its color changed uniformly to brown. The

Table 1—Data of instrumental analyses for methyl- and acetyl derivatives of compound (1)

	1,6-anhydro-2-0-acetyl-3,4-0-furfurylidene-β-D-galactopyranose	1,6-anhydro-2-0-methyl-3,4-0-furfurylidene-β-D-galactopyranose
Melting point	160–161°C	—
UV (methanol) λ_{max}	218 nm	221 nm
MS m/e	282(M, 3), 222(7), 181(11), 167(8), 147(16), 139(21), 126(9), 115(10), 85(13), 81(17), 69(18), 57(13), 44(10), 43(100), 42(9)	254(M, trace), 181(62), 158(49), 125(23), 113(38), 112(60), 111(26), 97(100), 95(34), 87(58), 85(30), 82(34), 81(79), 69(26), 58(49), 57(57), 45(38), 41(38)
^{1}H-NMR ($CDCl_3$)	7.49 (1H, sm), 6.58 (1H, d), 6.40(1H, m), 5.79(1H, s), 5.43(1H, s), 5.06(1H, s), 4.55(3H, m), 4.09(1H, dm), 3.65(1H, tm), 2.14(3H, s, $-CH_3$)	7.49(1H, sm), 6.57(1H, d), 6.40(1H, m), 5.80(1H, s), 5.49(1H, s), 4.51(4H, m), 4.15(1H, dm), 3.64(1H, m), 3.50(3H, s, $-CH_3$)
High-resolution-MS	282.0742(found, 282.0740)	254.0755(found, 254.0789)

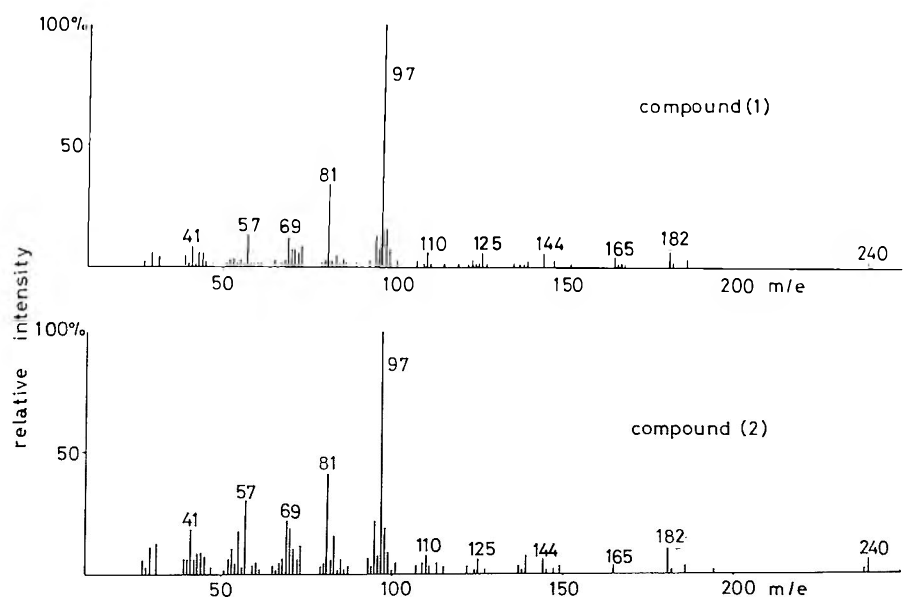

Fig. 2—Mass spectra of compound (1) and compound (2).

Table 2—Elemental analyses, melting points, ultraviolet spectroscopies and optical rotations obtained from compound (1) and compound (2)

	Compound (1)	Compound (2)
Elemental analysis	C% 55.33 H% 5.15	C% 54.97 H% 5.14
Melting point	123–124°C	123–124°C
UV(methanol) λ_{max} (ε)	221(2980) nm	221(3070) nm
Optical rotation	$[\alpha]_D$ +30° (c 0.4, methanol)	$[\alpha]_D$ −43° (c 0.6, methanol)

lactose pyrolysate was dissolved in 2L hot water, followed by addition of 10L methanol. The precipitate was removed by decantation. The supernatant was evaporated to dryness in a rotary evaporator and extracted with 2L hot ethyl acetate. The ethyl acetate-soluble fraction was evaporated to a syrup and redissolved in 20 mL ethyl acetate. Compound (**II**), which has an R_f 0.50 in solvent A, and a R_f 0.56 in solvent B was separated from the fraction by preparative TLC using solvents A and B, and extracted with ethyl acetate to yield 44.9 mg product.

2,4-Dinitrophenylhydrazone of 1

Compound (1) (2 mg) was reacted with 5 mL 1% 2,4-DNPhydrazine in 3% HCl-MeOH solution at room temperature for 30 min to give orange crystals. The solution remaining was treated with excess, 2,4-DNPhydrazine as a n-heptanal-DNPhydrazone. After addition of 10 mL water, n-heptanal and its DNPhydrazone were removed by extraction with ethyl ether, three times. The aqueous acidic fraction was neutralized with 1 N NaOH, evaporated to dryness, and then extracted with ethanol. Small amounts of syrup was obtained from the ethanol extract. This substance was subjected to GC of its TMS ether and to TLC using solvent E.

Methyl- and acetyl derivative of 1

The acetylation of 1 was carried out with acetic anhydride and pyridine in the usual way (Wolfrom and Thompson, 1963). The acetate (3) was extracted three times with chloroform and crystallized by evaporation of chloroform. The methylation of 1 was performed by the methods of Hakomori (1964) and the methylate (4) was purified further by silica gel chromatography on a column of Wakogel S-1. The alditol acetate derivative (5) was prepared from (4) by the methods of Stellner et al. (1973).

Preparation of 1 and 2 by the condensation of levogalactosan with furfural

The mixture of levogalactosan (1g) and furfural (10 mL) in a test tube was heated at 175°C for 2 hr in the presence of 10 drops conc nitric acid. The brown sample was poured into 600 mL water and extracted with 300 mL ethyl acetate. The extract was evaporated to a syrup in a rotary evaporator and dissolved in 10 mL methanol. The methanol solution was charged on preparative TLC using solvent D. The compounds (1) and (2) which have R_f 0.34 and 0.43, respectively, were extracted with ethyl acetate on the plates and crystallized from hexane-ether (1:1) and (2:1), respectively. Recrystallization of both gave colorless needles.

RESULTS & DISCUSSION

Identification of (1) in lactose pyrolysate

The UV maximum absorption in (1) at 221 nm (ε 2980) showed the presence of a conjugated double bond. The IR

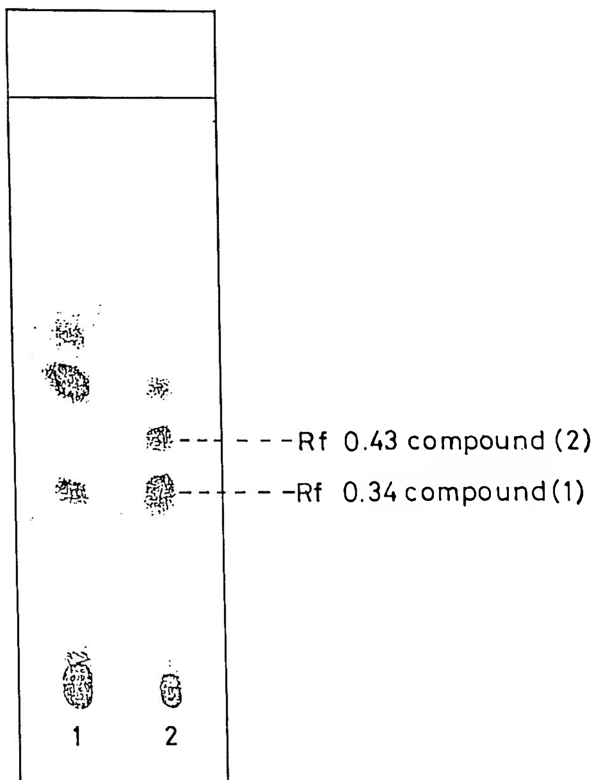

Fig. 3—TLC of the condensates formed from levogalactosan with furfural: 1. furfural (1 mL) and 100 mg levogalactosan heated at 175°C for 2 hr; 2. furfural (1 mL) and 100 mg levogalactosan heated at 175°C for 2 hr with two drops of conc nitric acid.

spectrum (Fig. 1) showed characteristic absorption bands due to OH (3480 cm^{-1}), conjugated C=C (1680 cm^{-1}), ether (1160, 1140, 1100, 1080, 1070, 1040, 1010, 1000, 980 cm^{-1}) and C-H of furan (820 cm^{-1}). The MS (Fig. 2) and the high resolution MS of (1) gave a molecular ion at m/e 240, and a molecular formula of $C_{11}H_{12}O_6$, respectively.

The reaction of (1) with 2,4-DNPhydrazine in HCl-MeOH solution gave orange needles. The R_f value of TLC, VIS and MS of this compound were identical with an authentic 2,4-DNPhydrazone of furfural. On the other hand, a component remaining in the DNPhydrazine-treated solution was identified as levogalactosan by comparison of the TLC and GC of its TMS ether with an authentic sample. These results indicated the presence of furfural and levogalactosan moieties in (1).

It was shown that the acetate (3) and the methylate (4) had one acetyl group and one methyl group, respectively, by their MS molecular ions and signals due to methyl groups in ^{1}H-NMR (Table 1).

The alditol acetate derivative obtained from (4) was identified as 2-O-methyl-1,3,4,5,6-O-pentaacetylgalactitol-1-^{2}H by comparison of the MS pattern with that of an authentic sample.

From the interpretation of all data, therefore, (1) was identified as 1,6-anhydro-3,4-O-furfurylidene-β-D-galactopyranose.

Detection of (2) in lactose pyrolysate

A trace amount of (2) which had a R_f value of 0.43 in TLC with solvent D was detected in the ethyl acetate extracts of lactose pyrolysate by TLC.

Synthesis of (1) and the stereoisomer (2) by condensation of furfural and levogalactosan

The thin-layer chromatogram of the mixture of furfural and levogalactosan heated at 175°C for 2 hr are shown in Fig. 3. Two spots with R_f values of 0.34 (compound 1) and 0.43 (compound 2), respectively, were detected in the sample heated in the presence of two drops conc nitric acid; compound (2) was not detected in the sample heated without nitric acid. By preparative TLC, 191.6 mg (1) and 100 mg (2) were isolated from 1g of the heated sample and crystallized from hexane-ether. As shown in Table 2, they were identical in analytical calculation of elemental analysis, melting point, absorption maximum and molecular absorbance in UV spectrum. The numerical value of optical rotation was reverse in direction of the rotation. Both IR absorption patterns (Fig. 1) were analogous except for bands at 2900 cm^{-1} and 1400 cm^{-1} which were due to C-H stretching and C-H bending, respectively. The MS patterns were essentially identical. From these data, it was deduced that (1) and (2) are stereoisomeric with each other at the acetal carbon atom. The ^{1}H-NMR spectra and assignments of their resonance signals are given in Fig. 4. It was deduced that H-6′ proton of (2) is close to H-3 and H-4, because the signal due to H-6′, which occurred at δ 5.77 in (1) shifted to δ 6.31 in (2). The signal shifts due to H-4 and H-3′ in (1), which occurred at δ 4.53 and δ 6.67, respectively,

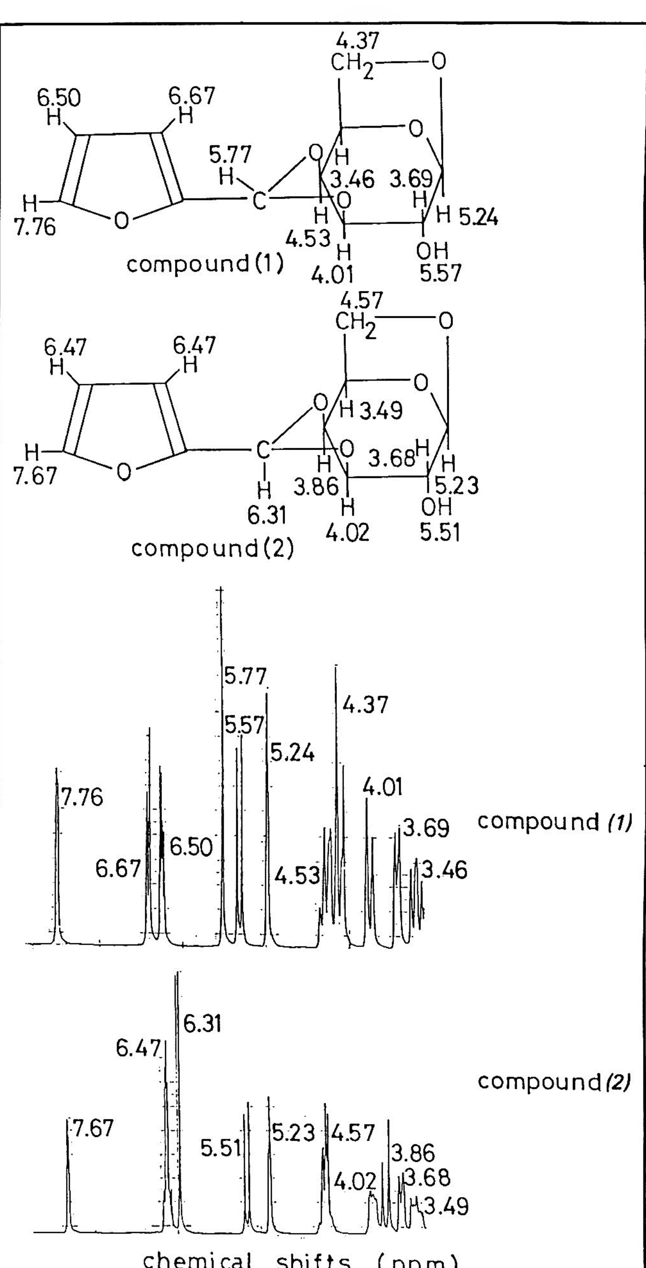

Fig. 4—^{1}H-NMR of compound (1) and compound (2) recorded in dimethyl sulfoxide - d_6 and the assignments of their signals.

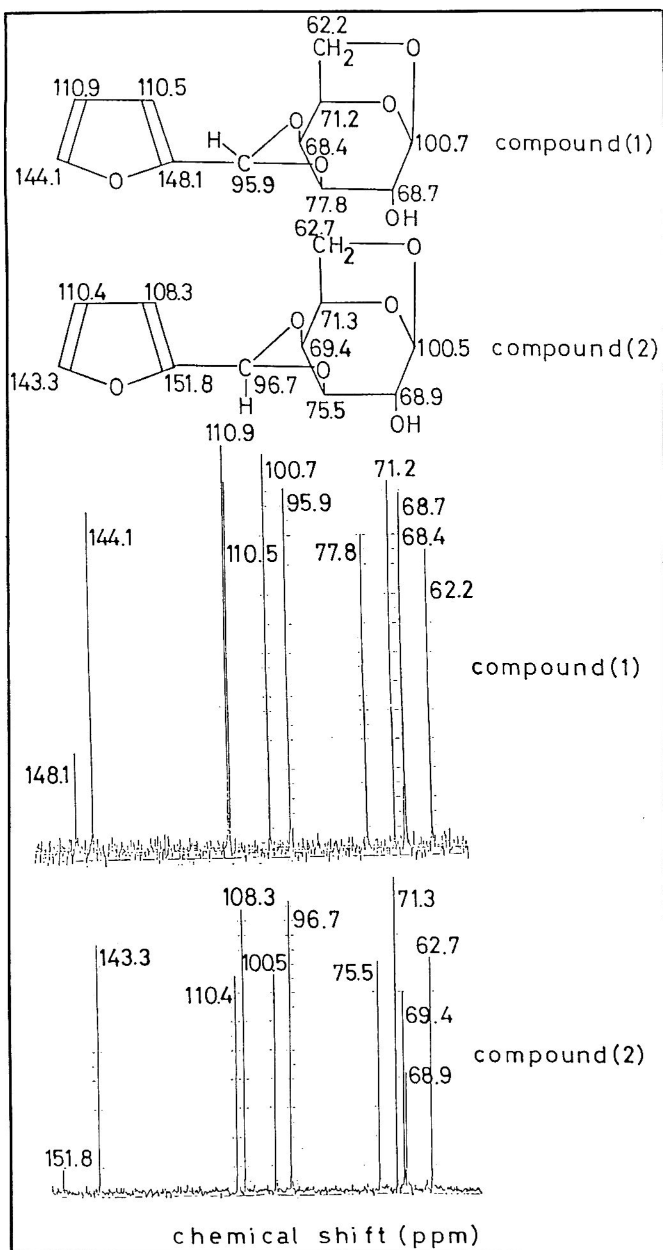

Fig. 5—^{13}C-NMR of compound (1) and compound (2) recorded in dimethyl sulfoxide - d_6 and the assignments of their signals.

at a lower field than that of (2) were presumed to be due to the difference in molecular configuration. From these interpretations, (1) and (2) were assumed to be identified as the trans- and cis-isomers of 1,6-anhydro-3,4-O-furfurylidene-β-D-galactopyranose, respectively. The ^{13}C-NMR spectra are given in Fig. 5.

Several furfurylidene derivatives of methyl α-glucoside, galctoside, mannoside and methyl β-glucoside were synthesized by Bredereck (1935, 1937) and Bredereck and Papademetriu (1937). The compounds (1) and (2) are novel furfurylidene derivatives. The formation of cyclic acetal such as (1), (2) and 1,6-anhydro-3,4-O-[5-(hydroxymethyl)-2-furfurylidene]-β-D-galactopyranose from sugars may be accelerated by heating at a low moisture content. Such analogous reactions would occur in baking of intermediate moisture foods which contain a large amount of sugars.

The stereoisomerisms in the acetal carbon atoms of furfurylidene derivatives have not been reported previously. The formation of (2) by condensation of furfural with levogalactosan may occur only with a catalyst. A trace amount of (2) was formed in pyrolysis of lactose, indicating that some compounds derived from lactose acted as catalyst to form (2).

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Near Infrared Spectrophotometric Determination of Individual Sugars in Aqueous Mixtures

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ABSTRACT

Near infrared region (NIR) spectrophotometry was used to determine concentrations of glucose, fructose and sucrose in aqueous mixtures at 10, 25, and 40% total sugars. Calibration equations and predicted individual concentrations in each total sugar level are reported. The standard errors of prediction varied between 0.35 and 0.69 and confidence limits ranged from ± 0.7 to ± 1.1%. In developing a single equation for predicting individual sugars in solutions, suitable for use over a range of total sugar concentrations, spectral data needed correction for sample density differences. However, at total sugar concentrations above 25%, accompanying wavelength shifts were of sufficient magnitude that a satisfactory single equation could not be developed. Equations for predicting individual sugars in solutions ranging from 10–25% total sugar concentrations were developed. For higher values of total sugars, appropriate calibrations were necessary.

INTRODUCTION

THE DETERMINATION of glucose, fructose, and sucrose content in fresh tissues is one of the chemical analyses carried out in evaluating the quality of a number of fruits (Lee et al., 1970). In some cases, it is sufficient to determine soluble solids refractometrically as a quality parameter. This index can be correlated with the taste of fruits since sweetness is related to sugars which are the major components of the soluble solids. In other cases it may be more appropriate to determine individual sugars, primarily glucose, fructose and sucrose. The procedures currently used for these analyses are destructive and time consuming (Lee et al., 1970).

For these reasons, in the last decade research has been carried out on the nondestructive determination of individual simple sugars in fruits and vegetables. Near infrared region (NIR) spectrophotometry appears to be one of the most promising nondestructive techniques. This approach is presently in wide use in determining chemical constituents and quality parameters in agricultural products, as noted in a recent review by Polesello and Giangiacomo (1983). However, there are not many published reports of determinations of sugars with this approach. McClure et al. (1977) determined total reducing sugars in dried, cured tobacco by direct spectrophotometric analysis. Dull et al. (1978) reported on preliminary studies on the measurement of individual sugars in fresh fruit tissue by NIR analysis, pointing out that the strong water absorption bands in this region of the spectrum make the determination of sugars difficult. Giangiacomo et al. (1981) demonstrated the possibility of using NIR spectrophotometry to measure individual sugars in dry mixtures containing other components which represented the composition of dried apples. The application of this procedure to aqueous solutions was not satisfactory. Later, Dull and Giangiacomo (1984) reported on the determination of glucose, fructose and sucrose individually in aqueous solutions in a wide range of concentrations by using an appropriate narrow range of wavelengths that enabled the use of a longer cell path. Lanza and Li (1984) applied NIR spectrophotometry to the determination of total sugars in fruit juices. They were able to determine total sugars in 11 types of fruit juices. However, they concluded that under the conditions of their experiment, it was not possible to determine individual sugars with acceptable accuracy or precision.

The objective of this work was to determine glucose, fructose and sucrose individually in aqueous mixtures in a range of total sugar concentrations of 10–40% weight/weight (w/w). This research represents a further contribution to the nondestructive determination of the three sugars in fruits and vegetables.

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MATERIALS & METHODS

Sample preparation

Three separate sets of samples were prepared for calibration purposes. In each set, the total sugar concentration was maintained constant at 10, 25, or 40% on a (w/w) basis. The ranges were chosen to evaluate the adequacy of the method for application to samples ranging from fresh fruit to processed products with high sugar concentrations. The (w/w) basis was used for greater accuracy of sample preparation. In each set, fifteen samples were prepared with each of the sugars (glucose, fructose, and sucrose) ranging from low to high values of concentration for each and in different ratios with the other two. It was reasoned that this approach to selecting ratios of individual sugars and ranges of total concentrations would provide as severe a test as possible for this method. Each solution was replicated to obtain 30 samples per set. These samples were designated as the calibration sets. A second group of three sets of solutions was prepared using the same total sugar concentrations but with the individual sugars differing from the specific concentrations in the calibration sets. In each set 15 samples were prepared and 5 of them were replicated to obtain a total of 20 samples per set. These samples were designated as the prediction sets.

The term "actual concentration" refers to the sugar concentration in a specific sample as prepared. "Predicted concentration" refers to a value computed from calibration equations.

The sugars used in this work were obtained from Sigma Chemical Company (St. Louis, MO) and were analyzed for purity by means of high performance liquid chromatography and refractive index. Double distilled deionized water was used in preparation of all solutions.

Spectral data acquisition and processing

Transmittance (T) measurements on the solutions were made at 24°C with a modified SpectroComputer (Pacific Scientific, Silver Springs, MD), a computerized multipurpose spectrophotometer similar to that described by Rosenthal et al. (1976). The sample cell used in this work was constructed with an aluminum body and quartz end plates. The path length was kept constant at 2.74 mm by using the appropriate spacers between the end plates. The empty cell was used as a reference standard. The wavelength range investigated in this work was 1550–1850 nm and was scanned at 10 nm per second. The monochromator slit was set at 2 mm, which gave an effective band pass of 7 nm. For each spectrum, 1000 data points were collected with a wavelength increment of 0.3 nm between data points. The spectral data were recorded as log 1/T. The instrument noise level was 0.0015 OD units over the entire range studied. To reduce the impact of this high noise level, each sample was scanned five times and the scans averaged to give a single spectrum. The spectrum obtained was smooth by sequentially averaging 21 data points, starting with data points 1 to 21 and continuing to data points 979 to 1000.

Table 1—Parameters and standard error terms for calibration equations used to predict the concentration of individual sugars in aqueous mixtures at three different total sugar concentrations

	Glucose			Fructose			Sucrose		
	Total sugars			Total sugars			Total sugars		
	10%	25%	40%	10%	25%	40%	10%	25%	40%
λ_1 nm	1661	1696	1695	1685	1688	1687	1711	1703	1702
λ_2 nm	1712	1664	1665	1609	----	----	1759	----	1674
K_0	88.22	−74.22	−47.14	37.84	26.13	32.20	61.81	72.71	52.00
K_1	−83.14	114.30	167.12	−80.05	1403.5	1360.8	74.82	1696.3	−160.91
Standard error of calibration	0.303	0.309	0.586	0.258	0.353	0.392	0.378	0.454	0.443
Standard error of prediction	0.371	0.442	0.352	0.476	0.472	0.687	0.382	0.563	0.481
Correlation coefficient	0.988	0.999	0.998	0.991	0.999	0.999	0.981	0.998	0.999

Table 2—Predicted concentrations for individual sugars in aqueous mixtures with 10% (w/w) total sugars

Glucose			Fructose			Sucrose		
Actual	Predicted	Difference	Actual	Predicted	Difference	Actual	Predicted	Difference
1.50	1.28	−0.22	6.00	5.85	−0.15	2.50	3.51	1.01
2.00	1.78	−0.22	5.00	5.0	0.08	3.00	3.38	0.38
1.50	1.63	0.13	1.50	1.34	−0.16	7.00	7.06	0.06
3.00	3.15	0.15	2.00	1.87	−0.13	5.00	4.96	−0.04
1.50	2.16	0.66	2.50	2.72	0.22	6.00	6.30	0.30
2.50	2.40	0.10	6.00	5.60	−0.40	1.50	1.21	−0.29
7.00	7.16	0.16	1.50	1.48	−0.02	1.50	1.35	−0.15
5.00	4.98	−0.02	3.00	3.47	0.47	2.00	2.01	0.01
6.00	5.99	−0.01	2.50	2.49	−0.01	1.50	1.58	0.08
2.50	2.83	0.33	1.50	1.19	−0.31	6.00	5.95	−0.05
5.00	4.73	−0.27	2.00	2.00	0.00	3.00	3.31	0.31
1.50	2.06	0.56	7.00	6.87	−0.13	1.50	1.32	−0.18
3.00	3.17	0.17	5.00	4.78	−0.22	2.00	2.49	0.49
6.00	5.46	−0.54	1.50	2.23	0.73	2.50	1.96	−0.54
2.00	1.66	−0.34	3.00	3.24	0.24	5.00	4.75	−0.25
1.50	2.09	0.59	1.50	1.11	−0.39	7.00	7.07	0.07
5.00	4.44	−0.56	3.00	2.98	−0.02	2.00	2.30	0.30
2.50	2.51	0.01	1.50	0.01	−1.49	6.00	6.59	0.59
1.50	1.48	−0.02	7.00	6.85	−0.15	1.50	1.49	−0.01
2.00	2.01	0.01	3.00	2.45	−0.55	5.00	5.28	0.28
r 0.984			0.976			0.986		

r = correlation coefficient between actual and predicted values.

Table 3—Predicted concentrations for individual sugars in aqueous mixtures with 25% (w/w) total sugars

Glucose			Fructose			Sucrose		
Actual	Predicted	Difference	Actual	Predicted	Difference	Actual	Predicted	Difference
2.00	2.78	0.78	21.00	20.58	−0.42	2.00	1.14	−0.86
5.00	5.26	0.26	3.00	3.58	0.58	17.00	16.33	−0.61
13.00	13.10	0.10	4.00	4.03	0.03	8.00	7.42	−0.58
17.00	16.64	−0.36	3.00	3.26	0.26	5.00	5.30	0.30
4.00	3.43	−0.57	8.00	7.74	−0.26	13.00	13.23	0.23
2.00	2.32	0.32	2.00	1.89	−0.11	21.00	20.29	−0.71
8.00	7.91	−0.09	13.00	12.46	−0.54	4.00	3.84	−0.16
8.00	7.76	−0.24	4.00	3.61	−0.39	13.00	12.41	−0.59
17.00	16.65	−0.35	5.00	5.15	0.15	3.00	3.44	0.44
21.00	22.04	1.04	2.00	2.38	0.38	2.00	1.03	−0.97
5.00	4.66	−0.34	17.00	17.48	0.48	3.00	2.70	−0.30
4.00	3.67	−0.33	13.00	12.97	−0.03	8.00	8.31	0.31
3.00	3.11	0.11	5.00	4.66	−0.34	17.00	17.05	0.05
13.00	13.20	0.20	8.00	7.59	−0.41	4.00	3.45	−0.55
3.00	3.39	0.39	17.00	16.38	−0.62	5.00	4.66	−0.34
8.00	7.78	−0.22	13.00	12.73	−0.27	4.00	3.74	−0.26
21.00	20.61	−0.39	2.00	2.10	0.10	2.00	2.27	0.27
4.00	3.92	−0.08	8.00	7.90	−0.10	13.00	12.42	−0.58
3.00	3.60	0.60	17.00	16.33	−0.67	5.00	4.03	−0.97
3.00	3.12	0.12	5.00	3.85	−1.15	17.00	16.74	−0.26
r 0.998			0.997			0.997		

r = correlation coefficient between actual and predicted values.

In the process of comparing the results for the three total sugar concentrations, consideration must be given to the differences in sample density. This is necessary since a fixed volume sample cell was used with solutions prepared on a (w/w) basis. The correction was made by dividing the spectral data by the density of each sample.

The analysis of the spectral data involved computation of second derivatives of spectra relative to changes in component concentrations. The procedure and computer programs described by Norris and Massie (1981) were used in this work. A detailed explanation of the data processing procedure is described by Dull and Giangiacomo (1984). The performance of this analytical method was evaluated by estimation of the correlation coefficient (r) between actual sugar concentra-

Table 4—Predicted concentrations for individual sugars in aqueous mixtures with 40% (w/w) total sugars

Glucose			Fructose			Sucrose		
Actual	Predicted	Difference	Actual	Predicted	Difference	Actual	Predicted	Difference
22.00	22.03	0.03	4.00	3.85	−0.15	14.00	13.69	−0.31
4.00	4.38	0.38	14.00	14.19	0.19	22.00	21.60	−0.40
14.00	14.14	0.14	22.00	22.32	0.32	4.00	3.28	−0.72
15.00	14.64	−0.36	7.00	6.21	−0.79	18.00	17.94	−0.06
7.00	7.48	0.48	18.00	17.86	−0.14	15.00	14.53	−0.47
18.00	17.50	−0.50	15.00	15.02	0.02	7.00	7.11	0.11
13.00	13.03	0.03	13.00	11.96	−1.04	14.00	14.59	0.59
14.00	13.94	−0.06	13.00	13.55	0.55	13.00	12.23	−0.77
13.00	13.04	0.04	14.00	13.90	−0.10	13.00	12.43	−0.57
3.00	3.76	0.76	18.00	17.60	−0.40	19.00	18.69	−0.31
18.00	17.50	−0.50	19.00	19.48	0.48	3.00	2.30	−0.70
19.00	19.03	0.03	3.00	2.07	−0.93	18.00	18.24	0.24
2.00	2.27	0.27	28.00	28.63	0.63	10.00	10.16	0.16
28.00	27.84	−0.16	10.00	10.46	0.46	2.00	1.67	−0.33
10.00	9.81	−0.19	2.00	0.98	−1.02	28.00	27.90	−0.10
14.00	13.77	−0.23	22.00	22.03	0.03	4.00	4.41	0.41
19.00	19.00	0.00	3.00	1.86	−1.14	18.00	18.67	0.67
14.00	14.47	0.47	13.00	12.11	−0.89	13.00	12.47	−0.53
10.00	10.38	0.38	2.00	0.88	−1.12	28.00	27.63	−0.37
7.00	7.29	0.29	18.00	18.25	0.25	15.00	14.73	−0.27
r 0.999			0.998			0.998		

r = correlation coefficient between actual and predicted values.

Table 5—Predicted concentrations for individual sugars in aqueous mixtures with 10% (w/w) total sugars using the combined calibration data for 10 and 25% concentrations

Glucose			Fructose			Sucrose		
Actual	Predicted	Difference	Actual	Predicted	Difference	Actual	Predicted	Difference
1.50	0.80	−0.70	6.00	5.70	−0.30	2.50	3.54	−0.95
2.00	2.28	0.28	5.00	4.90	−0.10	3.00	2.03	−0.97
1.50	1.48	−0.02	1.50	1.48	−0.02	7.00	6.51	−0.49
3.00	3.58	0.58	2.00	1.63	−0.37	5.00	3.72	−1.28
1.50	1.42	−0.08	2.50	3.01	0.51	6.00	6.02	0.02
2.50	1.83	−0.67	6.00	5.12	−0.88	1.50	0.82	−0.68
7.00	5.96	−1.04	1.50	1.50	−0.00	1.50	0.85	−0.65
5.00	3.93	−1.07	3.00	3.28	0.28	2.00	2.07	0.07
6.00	6.01	0.01	2.50	2.41	−0.09	1.50	0.28	−1.22
2.50	2.88	0.38	1.50	1.30	−0.20	6.00	4.76	−1.25
5.00	4.21	−0.79	2.00	2.25	0.25	3.00	3.66	0.66
1.50	1.77	0.27	7.00	6.25	−0.75	1.50	0.31	−1.19
3.00	3.05	0.05	5.00	4.51	−0.49	2.00	1.54	−0.46
6.00	5.11	−0.89	1.50	2.38	0.88	2.50	1.96	0.54
2.00	1.68	−0.32	3.00	3.52	0.52	5.00	3.99	−1.01
1.50	1.78	0.28	1.50	1.16	−0.34	7.00	6.25	−0.75
5.00	4.25	−0.75	3.00	3.06	0.06	2.00	1.43	−0.57
2.50	3.09	0.59	1.50	−0.11	−1.61	6.00	5.41	−0.59
1.50	0.50	−1.00	7.00	7.06	0.06	1.50	1.61	0.11
2.00	1.36	−0.64	3.00	2.45	−0.55	5.00	4.37	−0.63
r 0.951			0.959			0.954		

r = correlation coefficient between actual and predicted values.

tions and spectral data and by computation of standard errors of calibration (SEC) and standard errors of prediction (SEP).

RESULTS & DISCUSSION

THE SPECTRAL DATA for each calibration set were subjected to regression analysis for the development of calibration equations for each sugar in each of the three total sugar concentrations. The parameters for each set were optimized to fit the equation:

$$\text{CONC} = K_0 + K_1 \frac{2^{nd} \text{ Der log } 1/T_{\lambda_1}}{2^{nd} \text{ Der log } 1/T_{\lambda_2}}$$

where: K_0 = regression constant (intercept); K_1 = regression constant (slope); λ_1 = nominal wavelength for numerator second derivative; λ_2 = nominal wavelength for denominator second derivative.

The results are reported in Table 1. In some cases, a better calibration equation was obtained without the use of the denominator term. The calibration equations were then applied to the corresponding prediction data sets for the computation of the individual sugar concentrations. The predicted values for each set, compared with the actual values, are reported in Tables 2, 3 and 4. The calibration correlation coefficients varied from 0.981–0.999 and the SEP from 0.352–0.687. The 95% confidence limits ranged from ± 0.7 to ± 1.1%. In considering the accuracy of this method, the bias needs to be examined. If the algebraic sum of the differences between the actual and predicted values equals zero, there is no bias. For glucose, fructose and sucrose at all levels of total sugars, the bias ranged from +0.12 to −0.31. Since each sugar in each concentration range is predicted with a regression equation developed for specific conditions (Table 1), differences in individual biases could be expected. The bias was taken into consideration in developing the confidence limits as presented. The consistency of the computer selected wavelengths from which spectral data were obtained to determine each sugar suggested that a single equation might be used to determine each sugar component in the entire range of 10–40% total sugars. Data for 90 calibration samples were merged, and new calibration equations were developed. The standard errors (SEC and SEP) for these equations were considered too large to be acceptable.

A plot of the second derivative spectra for three solutions

Table 6—Predicted concentrations for individual sugars in aqueous mixtues with 25% (w/w) total sugars using the combined calibration data for 10 and 25% concentrations

Glucose			Fructose			Sucrose		
Actual	Predicted	Difference	Actual	Predicted	Difference	Actual	Predicted	Difference
2.00	3.03	1.03	21.00	20.75	−0.25	2.00	1.36	−0.64
5.00	5.77	0.77	3.00	3.43	0.43	17.00	17.34	0.34
13.00	13.29	0.29	4.00	4.74	0.74	8.00	8.00	0.00
17.00	16.72	−0.28	3.00	3.22	0.22	5.00	5.53	0.53
4.00	4.08	0.08	8.00	7.92	−0.09	13.00	13.65	0.65
2.00	2.49	0.49	2.00	2.09	0.09	21.00	20.52	−0.48
8.00	8.52	0.52	13.00	12.67	−0.33	4.00	3.36	−0.64
8.00	8.70	0.70	4.00	3.87	−0.13	13.00	12.76	−0.24
17.00	17.31	0.31	5.00	5.41	0.41	3.00	3.88	0.88
21.00	22.06	1.06	2.00	2.66	0.66	2.00	1.27	−0.73
5.00	4.68	−0.33	17.00	17.48	0.48	3.00	2.57	−0.43
4.00	3.88	−0.12	13.00	13.22	0.22	8.00	8.50	0.50
3.00	3.06	0.06	5.00	4.69	−0.31	17.00	16.89	−0.11
13.00	13.50	0.50	8.00	7.88	−0.12	4.00	3.51	−0.49
3.00	3.39	0.39	17.00	16.46	−0.54	5.00	5.12	0.12
8.00	8.59	0.59	13.00	12.78	−0.22	4.00	4.63	0.63
21.00	20.77	−0.23	2.00	1.80	−0.20	2.00	1.68	−0.32
4.00	4.50	0.50	8.00	8.31	0.31	13.00	13.33	0.33
3.00	4.37	1.37	17.00	16.53	−0.47	5.00	4.13	−0.87
3.00	4.42	1.42	5.00	4.62	−0.38	17.00	17.39	0.39
r 0.997			0.994			0.997		

r = correlation coefficient between actual and predicted values.

Table 7—Parameters and standard error terms for calibration equations developed from combined data for 10 and 25% calibration data sets

	λ_1 nm	λ_2 nm	K_0	K_1	SEC	SEP	Correlation coefficient
Glucose	1696	----	−121.32	−2009.4	0.580	0.666	0.995
Fructose	1687	1607	26.77	−42.61	0.362	0.489	−0.998
Sucrose	1699	1765	49.03	94.16	0.624	0.691	0.994

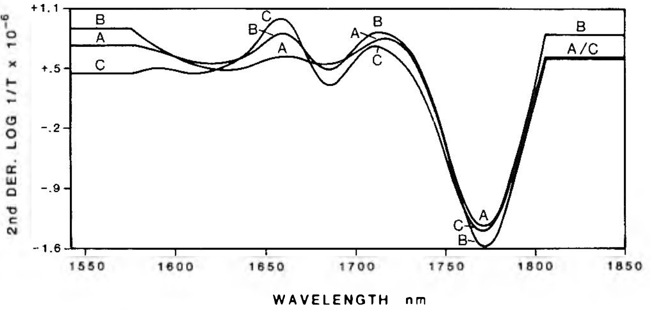

Fig. 1—Second derivative spectra of three sugar mixtures samples A, B, and C containing varying concentrations of glucose, fructose, and sucrose, respectively. A, 2.5, 2.5, 5.0; B, 6.25, 6.25, 12.5; C, 10, 10, 20%.

having different total sugar concentrations but the same ratios of the three components (Fig. 1) clearly shows that for increasing total sugar concentrations, there are wavelength shifts in the absorption bands, especially in the regions where the highest correlations between actual data and spectral data occur. We attribute these wavelength shifts to the changes in electronic energy levels of the sugars due to the increased competition for available water molecules at higher sugar concentrations. Because of these wavelength shifts, it was not possible to develop a single set of equations satisfactory for the complete range of total sugars from 10–40%. However, since the shift was greater at the higher total sugar concentration, an attempt was made to develop a single equation suitable for the 10 and 25% concentration ranges. The 60 calibration spectra were merged to create a new data set which was used to predict the sugar concentrations in the individual 10% and 25% data sets. The predicted values compared with the actual values are given in Tables 5 and 6. The computer-selected parameters for each sugar in the new calibration data set are reported in Table 7. The selected wavelengths for each sugar

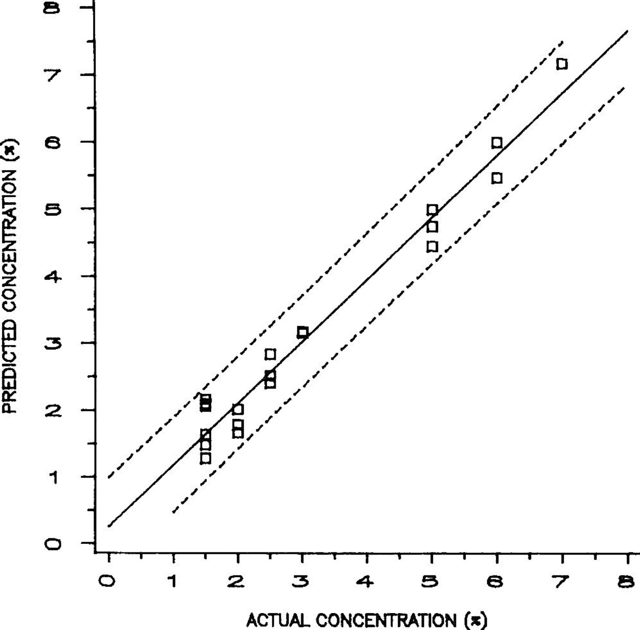

Fig. 2—Glucose concentrations (10% range) predicted with the 10% calibration data set and plotted against the actual concentrations. The solid line is the regression line (Y = 0.266 + 0.922 X; r = 0.984). The dotted lines are the 95% confidence limits.

are in very good agreement with those selected in the single sets (Table 1), even though for sucrose there is a slight shift toward lower wavelengths. It should be noted that the prediction equations developed with the combined calibration data differ from the equations developed with single calibration data sets. The application of these equations to the prediction data sets gave results which were sightly less accurate than the results with single sets of calibration data. The correlation

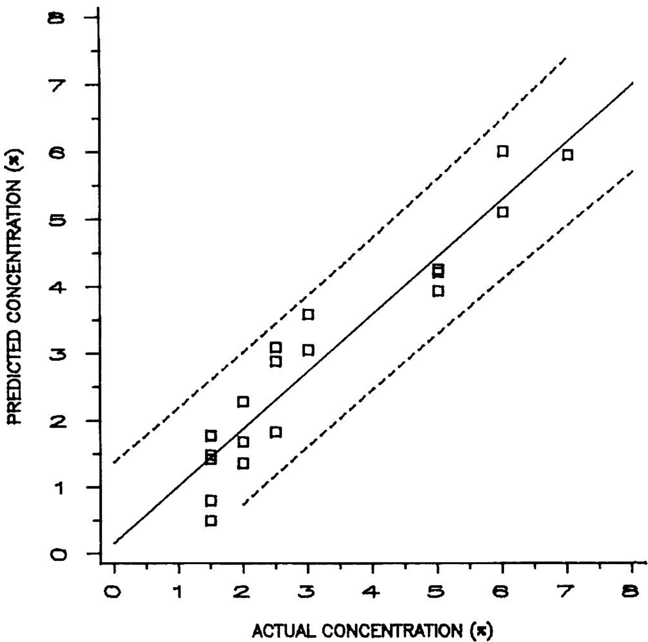

Fig. 3—Glucose concentrations (10% range) predicted with the combined 10 and 25% calibration data set and plotted against the actual concentrations. The solid line is the regression line ($Y = 0.167 + 0.856\,X$; $r = 0.904$). The dotted lines are the 95% confidence limits.

coefficients (0.994 to 0.998) were comparable to those for single set samples (Table 1), but the standard errors (SEC and SEP) were higher (0.50 to 0.68). The 95% confidence limits were also higher (± 0.8 to ± 1.4).

Another way to evaluate the accuracy of this method is to examine plots of predicted values against actual values. Scatter plots are presented for glucose (10% range) predicted with the single 10% calibration data set (Fig. 2) and the combined 10 and 25% calibration data set (Fig. 3). It is clear that the combined calibration data result in more scatter than the single set calibration data. The 95% confidence limits are ± 0.71% and ± 1.16 for Fig. 2 and 3, respectively. Under the conditions presented here, the lowest sugar concentration measured was 1.5%. Using a single calibration data set, concentration was predicted with confidence limits of 0.70, 0.98 and 0.79 for glucose, fructose and sucrose, respectively. With the combined calibration data set, these values were increased to ± 1.15, 1.21, and 1.38, respectively.

It can be concluded that the merger of the 10% and 25% calibration data into single calibration set enables the prediction of individual sugars in that range with slightly more error than a narrow range single set calibration. The decision to use a broad range calibration would depend upon the accuracy required for a specific application. These results can be compared with the determination of specific sugars in fruit by means of high performance liquid chromatography in which the concentrations are measured to the nearest 0.1%. The 10 and 25% concentration ranges have practical significance in that these ranges are frequently encountered in work with fruits and fruit products. The 40% concentration range has potential for application to food processes involving sugar syrups.

Instrument noise can be considered as the major factor in the standard errors. The noise level was reduced from 0.0015 to 0.001 OD units by repetitive scanning. This noise level equates to a sugar concentration of ± 0.6%. This accuracy was considered a satisfactory compromise between instrument limitations and a time consuming procedure of repetitive scanning.

The results obtained under our experimental conditions are adequate for us to state that individual sugars in aqueous mixtures can be determined nondestructively by NIR spectrophotometry. Assuming that the experimental error arising from instrument noise can be reduced with improved instrumentation, it is expected that the accuracy of the procedure will be similarly improved.

Further studies are planned on the possible interference of other chemical constituents in the determination of simple sugars by this method.

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Phase Transitions and Unfreezable Water Content of Carrots, Reindeer Meat and White Bread Studied using Differential Scanning Calorimetry

YRJÖ H. ROOS

ABSTRACT

Differential scanning calorimetry was used to measure the phase transitions and unfreezable water of carrots, reindeer meat, and white bread. The incipient melting point (T_{im}), incipient intensive melting point (T'_{im}), the onset temperature of melting (T_m), latent heat of melting (ΔH_m), specific heat (C_p) and enthalpy (ΔH) were determined from the melting curves. T'_{im}, T_m, ΔH_m and ΔH and the unfreezable water were found to be functions of moisture. The T_{im} temperatures were −39 °C, −33 °C, −40 °C; T'_{im}, −11.8 °C, −13.3 °C −17.3 °C; T_m, −3.4 °C, −3.1 °C, −12.2 °C for carrot, reindeer meat, and white bread, respectively. The unfreezable water was 8.3% for carrots, 15.1% for reindeer meat, and 22.5% for white bread, determined from ΔH_m, and 3.4%, 6.4% and 2.9%, determined from ΔH. The lowest water detectable from ΔH_m was 26.4% and from ΔH 3.6%.

INTRODUCTION

WATER is the major constituent of food and biological materials. The water in food is considered to be either bound or free. The definitions of these states of water are not exact but the amount of water bound is often defined to be the same as the amount of unfreezable water (Duckworth, 1971; Ross, 1978). Simatos et al. (1975a) found that the bound water does not freeze at normal temperatures.

The quality of freeze-dried materials is determined by the state of the water content at the freezing temperatures. Differential thermal analysis (DTA) is widely used in the studies of the state of water (Rey, 1960; Ito, 1970), and for measuring the unfreezable water of food materials (Duckworth, 1971; Parducci and Duckworth, 1972; Simatos et al., 1975a). Berlin et al. (1970) and Ross (1978) have used differential scanning calorimetry (DSC) to study the unfreezable water of biological materials. In these studies the fusion of ice is seen as an endothermic peak and the area under the peak is proportional to the amount of freezable water.

DTA and DSC can be used to determine glass transition temperatures (T_g), incipient melting temperatures (T_{im}), antemelting and the unfreezable water of food and biological materials (Guegov, 1981). This study was made to measure the melting behavior and unfreezable water of carrots, reindeer meat and white bread using differential scanning calorimetry.

MATERIALS & METHODS

THREE FOOD MATERIALS of different initial water content were used. These were carrots, reindeer meat and white bread. The carrots were bought from a local dealer, the reindeer meat was supplied by Poro ja Riista Oy (Rovaniemi), and the white bread was the product of a local bakery (Fazer). The initial water content of the materials was determined from weight loss at 105 °C for 18 hr.

A Mettler TA 3000 system DSC 30 differential scanning calorimeter was used. Samples, 2 — 15 mg, were prepared with a small knife just before analysis and enclosed in hermetically sealed aluminium pans (Mettler, 40 μL). An empty aluminium pan was used as a reference sample, and liquid nitrogen was used for cooling of the cell. To eliminate the enthalpy of the aluminium pan, a blank curve was determined for the temperature range of −100 °C to +50 °C. Water condensing was found in the measuring cell at temperatures below −100 °C which was avoided by using a nitrogen gas flush of 20–30 mL/min during calibration and measurements. This was not found to affect transition temperatures after calibration. The time constant for the temperature equilibration between the furnace and the sample was determined using indium by measuring the melting temperature with different heating rates from 1 °C/min to 10 °C/min. Heating rates up to 5 °C/min were found suitable and a heating rate of 2 °C/min was chosen. Temperature calibration was done with indium (mp 156.6 °C, Mettler standard), water (mp 0.0 °C ion exchanged, distilled) and n-hexane (mp −93.5 °C, Fluga AG, standard for gas chromatography, puriss. >99.7 %) using a heating rate of 2 °C/min as in the measurements. The heat flow was calibrated using the heat of fusion of indium (28.45 J/g). The calorimetric sensitivity (E) was determined using different heating rates and an average value of 236.12 points/mW was used. The energy changes in the samples were scanned for temperatures rising from −80 °C to +30 °C. At least four measurements were made for each material.

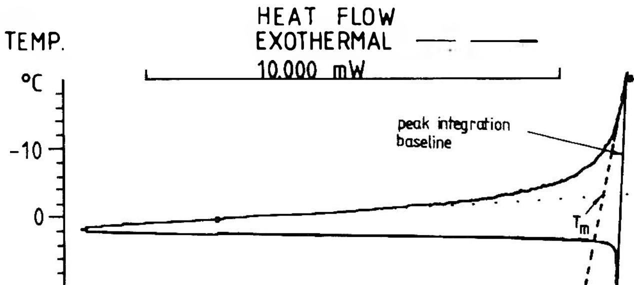

Fig. 1—Peak integration and determination of the temperature of onset of melting (T_m).

The incipient melting point (T_{im}) of the materials was determined from the melting curves by the method of Rey (1960). The incipient intensive melting point (T'_{im}) was determined from the enthalpy changes as the temperature above which a clear molten fraction was observed. The latent heat of melting (ΔH_m) was determined by integration of the peaks in the melting curve for temperatures ranging from −20 °C to +10 °C as shown in Fig. 1. ΔH_m was used to calculate the unfreezable water of the materials as described by Ross (1978). The onset temperature of melting (T_m) was determined from the melting curves as shown in Fig. 1. The specific heat (C_p) and enthalpy (ΔH) were determined for the temperature range −60 °C to +10 °C. The enthalpy was established as being O J/g at −60 °C. The unfreezable water was calculated from ΔH in the same way as from ΔH_m. A ΔH_m of 325 J/g and ΔH of 512 J/g were determined for water and used in calculations.

The correlation coefficients relating T_{im}, T'_{im}, ΔH_m, ΔH and moisture content (m) were calculated. The statistical significance of the correlation coefficients was checked using the

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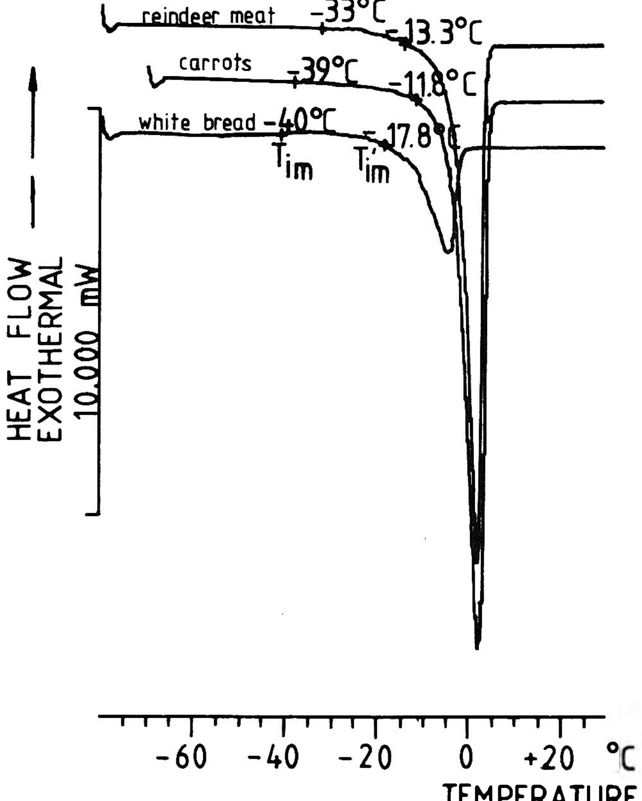

Fig. 2—The melting curves for carrots, reindeer meat and white bread.

Table 1—Moisture content, onset temperature of melting (T_m), latent heat of melting (ΔH_m) and enthalpy (ΔH) for the different materials

Material	Moisture %	T_m°C	ΔH_m J/g	ΔH J/g
Carrots	88.0	−3.4	259.3	432.9
Reindeer meat	74.9	−3.1	194.5	350.5
White bread	36.7	−12.2	46.2	172.9

t-test. Regression equations between m and T_m, ΔH_m, ΔH, and the frozen water content were determined.

RESULTS & DISCUSSION

THE MELTING CURVES for the materials are shown in Fig. 2. The melting of the ice in the materials studied began at low temperatures (−33 °C to −40 °C). T_m, ΔH_m, ΔH and moisture content of the materials are given in Table 1. The correlation coefficients and their statistical significance are given in Table 2, and the regression equations in Table 3.

The temperature of the incipient melting point (T_{im}) of reindeer meat (Fig. 2) is near the value for horse serum (−34.5 °C) as determined by Rey (1960) and that of plasma (−35 °C) as determined by Simatos et al. (1975b). The correlation between moisture (m) and the T_{im} of different materials was poor (Table 2). Simatos et al. (1975b) found that the incipient melting point of plasma was independent of the moisture content. It is therefore evident that T_{im} is a specific temperature for each material.

The specific sublimation temperature for ice in the freeze-drying process is between the incipient melting point and the incipient intensive melting point (Rey, 1964), and in this study ranged from −40 °C for white bread to −11.8 °C for carrots. It was also found that T'_{im} was a function of the moisture content; the lower it was, the lower was the moisture content (Table 2). Rey (1960) has pointed out that some materials must be freeze-dried at T_{im} or at a lower temperature.

Parducci and Duckworth (1972) reported that the lower the moisture content was, the lower the melting point and the broader the melting peak were. This study confirmed these conclusions, although the onset temperature for carrots was lower than that for reindeer meat (Table 1). The frozen and unfrozen water contents of the materials were calculated from ΔH_m and ΔH. The results are given in Fig. 3. The energy changes in the materials are shown in Fig. 4. Melting at temperatures between T_{im} and T'_{im} affected ΔH but not ΔH_m. The low temperatures for incipient melting confirmed that a part of the water in food was liquid at very low temperatures, and the amount of unfreezable water determined from the latent heat of melting was not completely accurate. In this study the unfreezable water of the materials was found to be a function of the moisture as shown in Fig. 3 and was 26.4%, if estimated from the regression equation of the moisture content and the latent heat of melting (Fig. 4). Riedel (1957) reported an unfreezable water content in meat of 26%, and Simatos et al. (1975b) reported 32% for plasma. This might be the critical water content at which no melting peak is observed in DTA and DSC curves. Duckworth (1971) determined the unfreezable water content of carrots to be 18.5–19.8%. In this study the unfreezable water content of carrots was 8.3 % (Table 4), determined from the latent heat of melting, and much higher than values previously determined using DTA (Duckworth, 1971). It was also found that, if the unfreezable water was determined from the latent heat of melting, it increased with decreasing moisture (Fig. 3). However, when the unfreezable water was determined from the enthalpy, the unfreezable water was independent of the moisture, and a straight line graph of total moisture against frozen water was obtained (Fig. 3). It was obvious that, at very low temperatures, almost all the water was in the solid state. The incipient melting at very low temperatures can be observed from the specific heat curves (Fig. 5). According to these observations there is a temperature at which the frozen water is the same as the moisture as shown in Fig. 3.

In freeze-drying, there are two phenomena determining structure, eutectic melting and collapse. Eutectic melting is possible in the ice phase, and collapse in the partially dry phase (MacKenzie, 1975). Because the incipient intensive melting point is a function of the moisture of the material, it is evident that collapse is the more important structure-determining factor in the freeze-drying of materials with high moisture, and that eutectic melting is more important for those with low moisture. Roos and Laine (1985) reported collapse in carrots during freeze-drying. This might have been caused by collapse, because the carbohydrate content of carrots is high, and the incipient intensive melting point of carrots, determined in this study, is high.

Table 2—Correlation coefficients relating incipient melting point (T_{im}), incipient intensive melting point (T'_{im}), onset temperature of melting (T_m), latent heat of melting (ΔH_m), enthalpy (ΔH) and moisture

	T_{im}	T'_{im}	T_m	ΔH_m	ΔH	Moisture
T_{im}	1	0.348	0.560*	0.333	0.323	0.364
T'_m		1	0.921***	0.979***	0.972***	0.986***
T_m			1	0.939***	0.914***	0.949***
ΔH_m				1	0.989***	0.997***
ΔH					1	0.990***

* significant at 5% level

*** significant at 0.1% level

Table 3—Regression equations between moisture content (m) and onset temperature of melting (T_m), latent heat of melting (ΔH_m), enthalpy (ΔH) and frozen water content. (n = 14)

Parameter (y_i)[a]	r	a	b
T_m	0.956	−18.650	0.187
ΔH_m	0.996	−110.887	4.193
ΔH	0.991	−18.533	5.095
Frozen H_2O	0.998	−1.439	0.982

[a] $y_i = a + bm$; r = correlation coefficient; a = constant; b = regression coefficient.

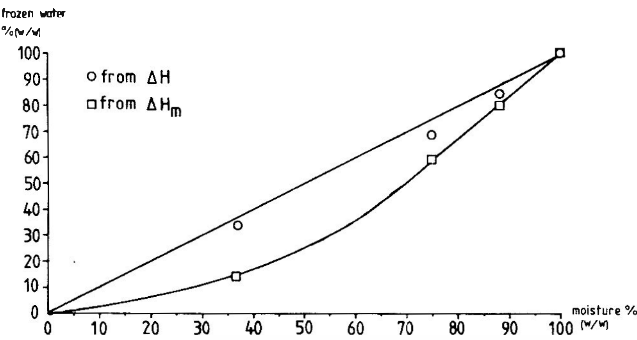

Fig. 3—Frozen water content versus total water content of water, carrots, reindeer meat and white bread, determined from latent heat of melting (ΔH_m) and enthalpy (ΔH).

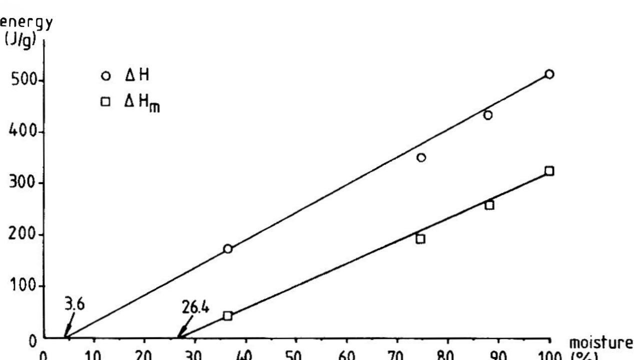

Fig. 4—Latent heat of melting (ΔH_m) and enthalpy (ΔH) of water, carrots, reindeer meat and white bread as function of moisture content.

Table 4—Frozen and unfrozen water content of the materials

	I[a]		II[b]	
Material	Frozen %	Unfrozen %	Frozen %	Unfrozen %
Carrot	79.7	8.3	84.6	3.4
Reindeer meat	59.8	15.1	68.5	6.4
White bread	14.2	22.5	33.8	2.9

[a] Frozen and unfrozen moisture calculated from ΔH_m
[b] Frozen and unfrozen moisture calculated from ΔH

The DSC method is simple and rapid for the determination of the melting-behavior of food. The results of the measurements can be used in the determination of the freeze-drying parameters for food and biological materials. The unfreezable water, determined from the latent heat of melting, was the amount present at freeze-drying temperatures and at the incipient intensive melting point. The exact unfreezable water should be determined at lower temperatures. One possible method is to determine this from the enthalpy changes in the different materials, because the energy changes in food and biological materials at low temperatures are mostly energy changes in their water content, and the phase transitions occur between the solid and liquid states.

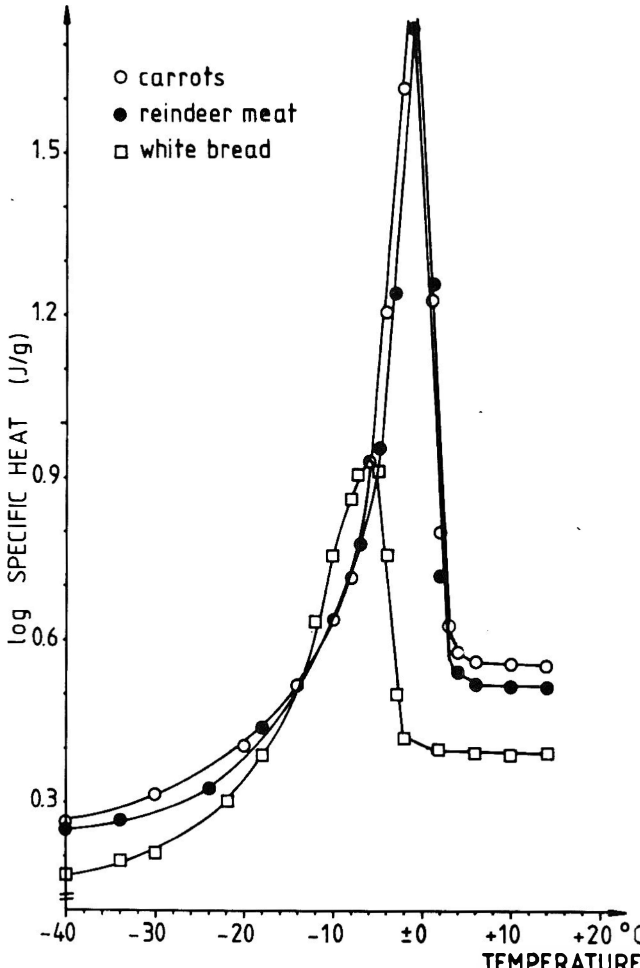

Fig. 5—Specific heat curves for carrots, reindeer meat and white bread.

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Mechanical Attrition Rate Measurements in Agglomerated Instant Coffee

J. MALAVE-LOPEZ and M. PELEG

ABSTRACT

Various size fractions of commercial agglomerated instant coffee were subjected to vertical and horizontal vibration at various frequencies. The resulting size reduction and its rate are described mathematically by a three parameter, two term, exponential decay model and a two parameter, nonexponential, model. The constants of these models were used to quantify the effects of particle size, vibration frequency and filling ratio on the agglomerates' attrition rate. The two models, despite their different mathematical structure, yielded a consistent account of the attrition kinetics.

INTRODUCTION

INSTANT COFFEE AGGLOMERATES being fragile and brittle have the tendency to disintegrate upon friction with other particles or impact with a solid surface. The main product of such an undesirable size reduction process, known as mechanical attrition, is fine particles. The presence of fines has an obvious adverse effect on the product appearance but it can also affect other physical properties, notably the bulk density and dispersibility. For this reason instant coffee manufacturers make a great effort to minimize mechanical attrition during processing and to remove (and recycle) those fines that are produced.

As in other size reduction operations, the extent and rate of instant coffee mechanical attrition depends on the material strength, the particles' physical structure and the mechanical conditions to which the particles are exposed. The latter, as evident from size distribution measurements, can affect not only the quantitative aspects of the attrition rate but also the breakage mechanism (Malave and Peleg 1986).

The most convenient form of data presentation for attrition studies is the attrition curve, which describes the disappearance of particles having the original size as a function of time. In many intentional size reduction operations the shape of the attrition curves (Fig. 1) can be characterized by a single term exponential decay model in the form of (e.g. Austin, 1972; Austin et al., 1981; Mika et al., 1967):

$$W(t) = \exp(-St) \quad (1)$$

where $W(t)$ is the momentary weight fraction of the particles retaining the original size after time t, and S a characteristic "rate" constant having units of time reciprocal. The validity of this model can be tested from the linearity of log $W(t)$ vs t relationship. It has been observed though (Fig. 2), that this simple model is inadequate for describing the mechanical attrition of instant coffee agglomerates having a size larger than about 16 mesh (Malave-Lopez et al., 1985b). For such agglomerates a two term exponential model was required, i.e.:

$$W(t) = A\exp(-S_1 t) + (1\text{-}A)\exp(-S_2 t) \quad (2)$$

where S_1 and S_2 are two "rate" constants. The constant A and the term 1-A according to this model represent the fractions of the material that disintegrate at the characteristic rates S_1 and S_2. (For convenience the presentation of data is in the form where $S_1 > S_2$).

As in the case of other decay processes, such as mechanical relaxation and electrostatic charge dissipation, (Peleg, 1980; Malave-Lopez and Peleg, 1985), the curves could also be fitted by a two parameter nonexponential model in the form (Malave-Lopez et al., 1985b):

$$W(t) = 1 - 1/(K_1 + K_2 t) \quad (3)$$

where $1/K_1$ is the initial attrition rate and $1/K_2$ the asymptotic weight fraction of the attrited material.

The parameters of Eq. (2) and (3) can be employed not only to characterize individual attrition curves but also to evaluate the effects of external conditions on the attrition process kinetics.

The objective of this work was to study the attrition process in agglomerated instant coffee and to quantify the effects of vibration, particle size and filling ratio in terms of the constants of Eq. (2) and (3).

MATERIALS & METHODS

COMMERICAL agglomerated instant coffee was gently sieved to obtain fractions with narrow size distribution. Samples of these fractions were subjected to vertical and horizontal vibration in a metal cell as described by Malave-Lopez et al. (1985a). The sample, after a predetermined time in vibration, was gently sieved to determine the weight fraction of the particles having the original size. All the tests were repeated with freshly prepared samples.

The attrition curves that resulted (Fig. 1) were fitted to the models outlined in Eq. (2) and (3) using the nonlinear regression program of the SPSS (Statistical Package for the Social Sciences). Comparison of the mean standard error in the fit of the two models showed that the two models were equal (Malave-Lopez et al., 1985b). The constants of these models were plotted as the function of the test conditions, namely the initial particle size, the vibration frequency, and the filling ratio.

RESULTS & DISCUSSION

Effect of particle size

The dependency of the constants of Eq. (2) and (3) on the agglomerates initial size in vibration is shown in Fig. 3 to 6. As could be expected, the susceptibility to breakage increased with the size in what appears to be a semi-logarithmic relationship between the rate parameters and the mesh size, i.e.

$$\log S_1 \text{ or } \log S_2 \text{ or } \log 1/K_1 \alpha \overline{D}_i \quad (4)$$

where $\overline{D}_i$ is the initial mean mesh size. The slope of these relationships is primarily determined by the external conditions such as the vibration intensity or the filling ratio (see below). One ought to notice that application of the two models does not yield exactly the same information on the attrition process. One of the main differences between the models is that the exponential model implies that *all* the original particles will disappear after a sufficiently long time since as $t \rightarrow \infty$ $W(t) \rightarrow 0$ while the nonexponential model allows for the existence of particles surviving the attrition process. This is because, according to Eq. (3), when $T \rightarrow \infty$, $W(t) \rightarrow 1\text{-}1/K_2$. (The term sur-

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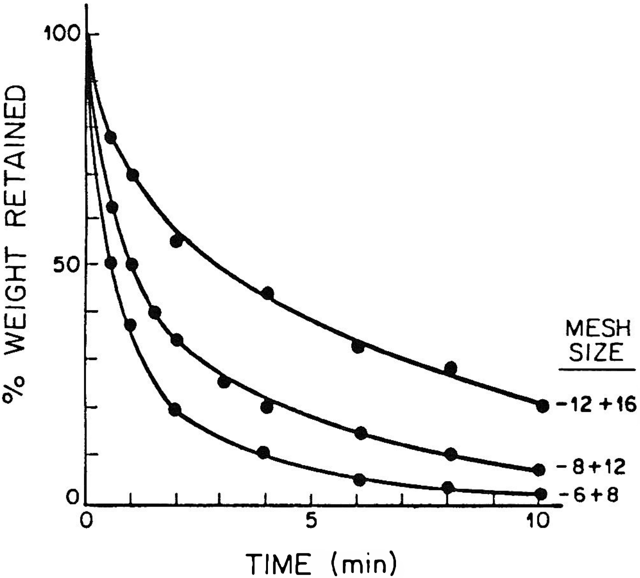

Fig. 1—Attrition curves of instant coffee agglomerates having different initial particle size.

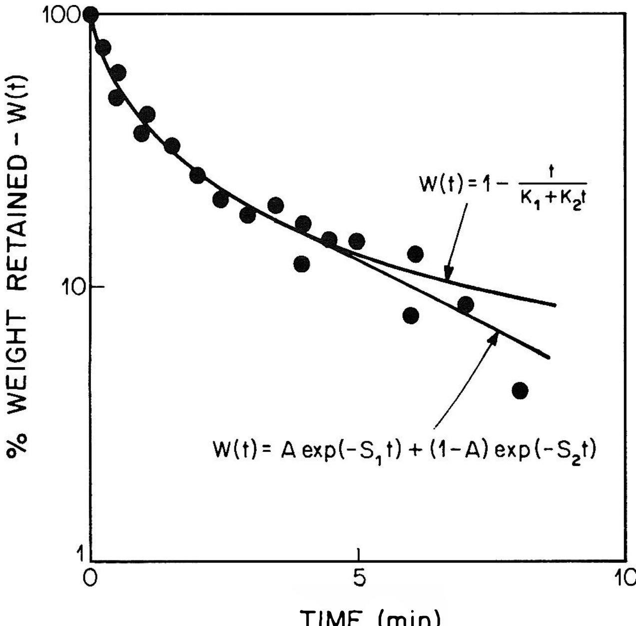

Fig. 2—Example of a semi-logarithmic presentation of an attrition curve of agglomerated instant coffee and a demonstration of the fit of two parameter and three parameter models. (A is a ratio coefficient, S_1 and S_2 rate characteristics, $1/K_1$ the initial rate and $1/K_2$ the asymptotic weight fraction of the attrited material.

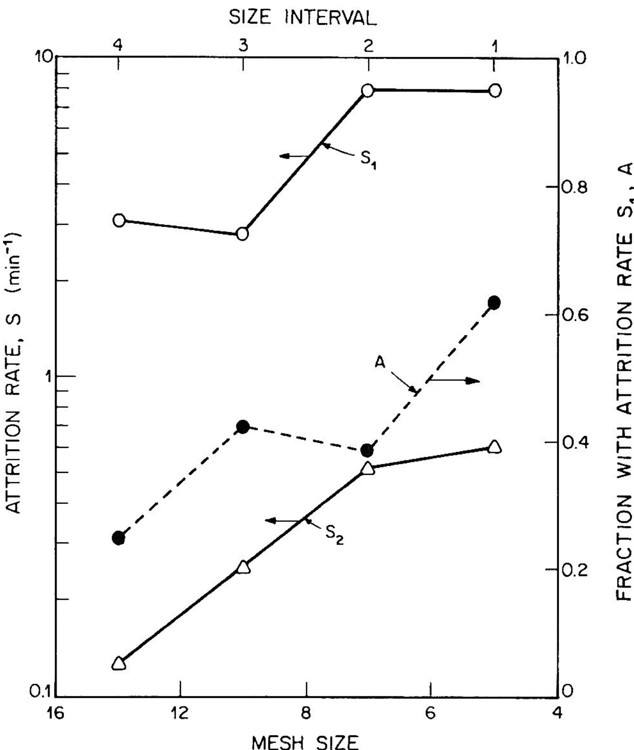

Fig. 3—Effect of the agglomerate size on the attrition parameters as determined by a three parameter exponential model (see Fig. 2). S_1 and S_2 and rate characteristics and A the fraction with an attrition rate S_1. (Vertical vibration 400 rpm).

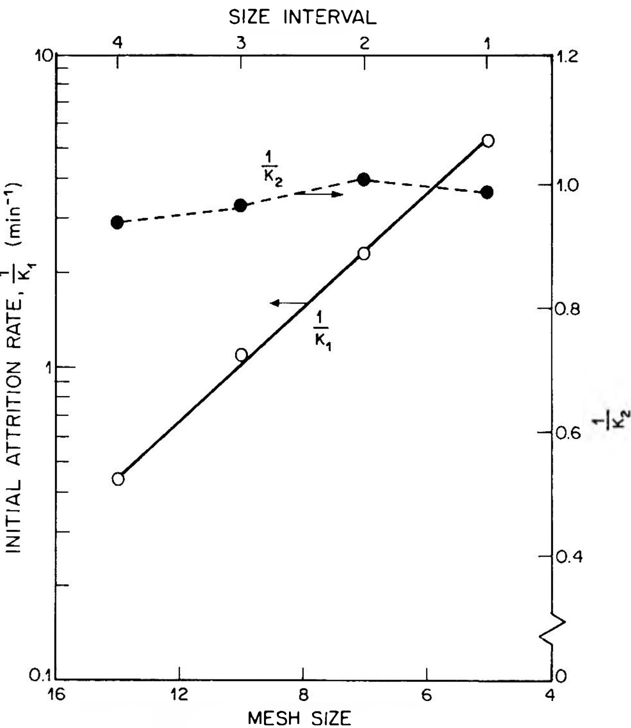

Fig. 4—Effect of the agglomerate size on the attrition parameters determined by a two parameter nonexponential model (see Fig. 2). $1/K_1$ is the initial rate and $1/K_2$ the asymptotic fraction of the attrited material. (Vertical vibration 400 rpm).

viving particles here does not mean that their size is not reduced at all. It only indicates that there can be a nonzero fraction of agglomerates whose size remains such that they can be captured between the two upper sieves).

It has been observed though, particularly for the larger agglomerates, (Fig. 4 and 6), that the values of $1/K_2$ were close to one indicating little or no remaining particles having the original size (when $1/K_2 = 1, W(\infty) = 0$). This means that as far as describing the attrition phenomenon, at least under the conditions of this work, the two models were in agreement despite their different mathematical formulation.

Analysis of the data presented in terms of the exponential model (Fig. 3 and 5) reveals that the magnitude of the rate constants S_1 and S_2 increased in unison. It was also observed (see figures) that the relative weight of the first decay term expressed by the magnitude of the constant A, increased almost linearly with the mesh size. This can be interpreted as a sign that large agglomerates not only disintegrate at a faster overall rate but also that an increasing proportion of the damage occurs

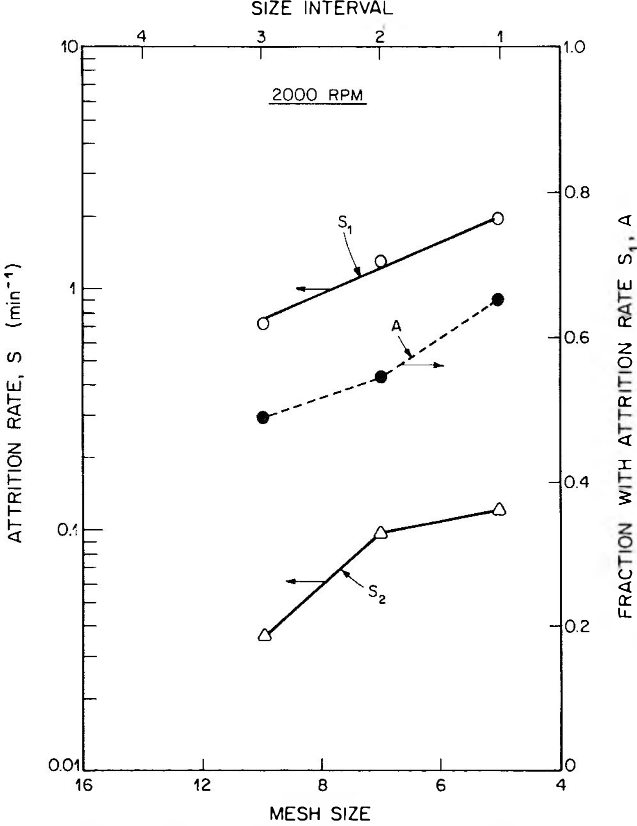

Fig. 5—Effect of the agglomerate size on the attrition parameters as determined by a three parameter exponential model (see Fig. 2). S_1 and S_2 are rate characteristics and A the fraction with an attrition rate S_1. (Horizontal vibration 200 rpm).

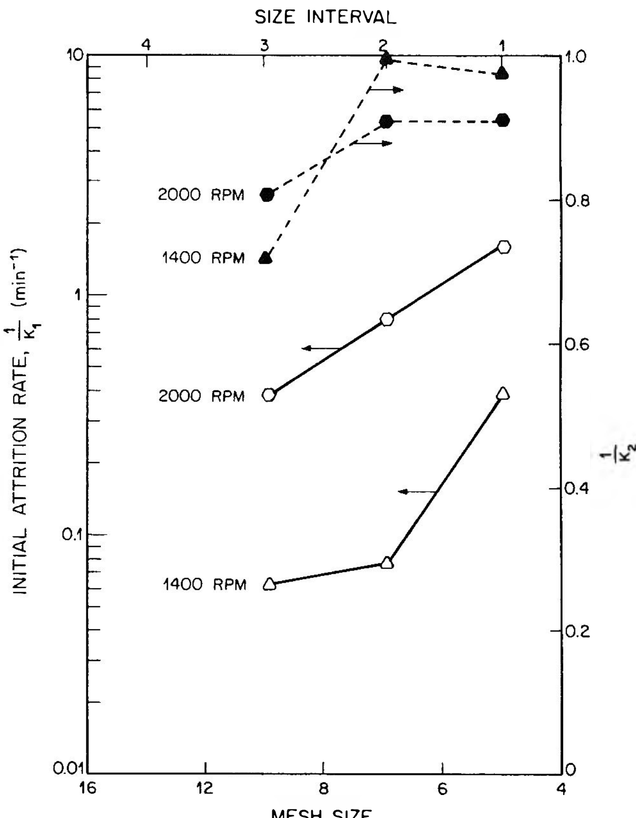

Fig. 6—Effect of the agglomerate size on the attrition parameters determined by a two parameter nonexponential model (see Fig. 2). $1/K_1$ is the initial rate and $1/K_2$ the asymptotic fraction of the attrited material. (Horizontal vibration 1400 and 2000 rpm).

within a shorter time after the exposure of the powder to vibration.

Vibration intensity

The effect of the experimental shaker's rpm and the vibrator's horizontal vibration frequency on the attrition rate constants are demonstrated in Fig. 7 to 9. Although admittedly the system's rpm or frequency is only an empirical and arbitrary intensity quantifier, the data can still be used to characterize general trends. As could be expected, an increased vibration frequency in either the horizontal or vertical direction induced higher attrition rate. The magnitude of the effect, not surprisingly, depended on the size of the agglomerate as demonstrated in Fig. 8. The agglomerates chance of survival also depended on the vibration frequency but as demonstrated in Fig. 9, only at low frequency was the attrition less than complete.

Under the test conditions that produced the data shown in the figure, the survival was practically nil when the agglomerates were exposed to frequencies higher than 1400 rpm. The slightly lower than one values of 1/K in some of the tests only reflect errors originating from the curve fitting procedure, (and as already mentioned, an asymptotic survival ratio of about 5–10%, even if real, is of no practical significance).

Effect of filling ratio

Results of attrition tests performed with partially filled cells are demonstrated in Fig. 10 and 11. As could be expected, the attrition rate decreased with the increase of the filling ratio. This was a result of the fact that at incomplete filling the agglomerates freedom of motion increases considerably. The latter allowed for higher accelerations, thus increasing the impact intensity with the container ends and other particles, and also for more intense interparticle friction. The magnitude of

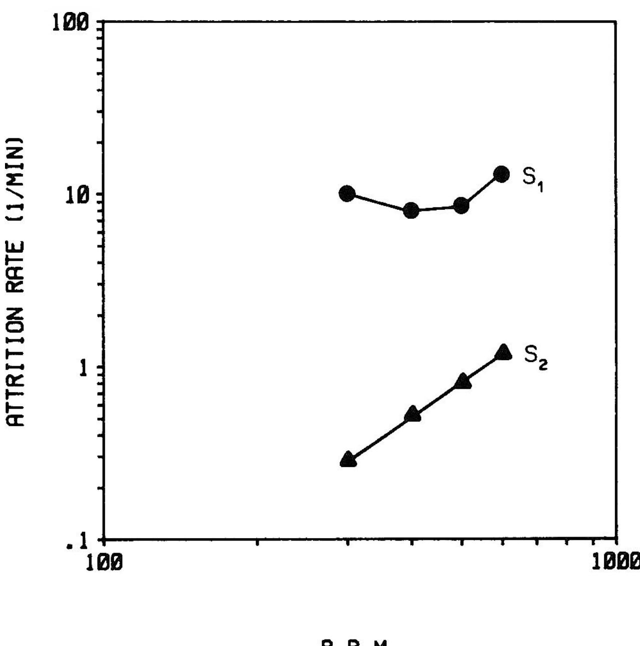

Fig. 7—Effect of the vertical vibration frequency on the attrition rate characteristics (S_1 and S_2) of agglomerated coffee as determined by a three parameter exponential model (Fig. 2).

the filling ratio effect depended on both the system vibration frequency and the size of the agglomerates. The data in Fig. 11 suggest though that the vibration frequency played a more dominant role, indicating that the increased impact and friction

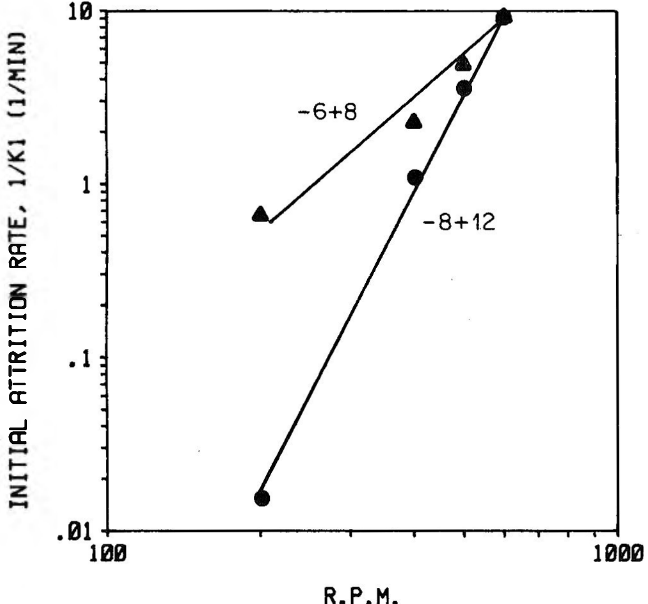

Fig. 8—Effect of the vertical vibration frequency on the initial attrition rate ($1/K_1$) of two mesh fractions of agglomerated instant coffee, determined by the two parameter non-exponential model (Fig. 2).

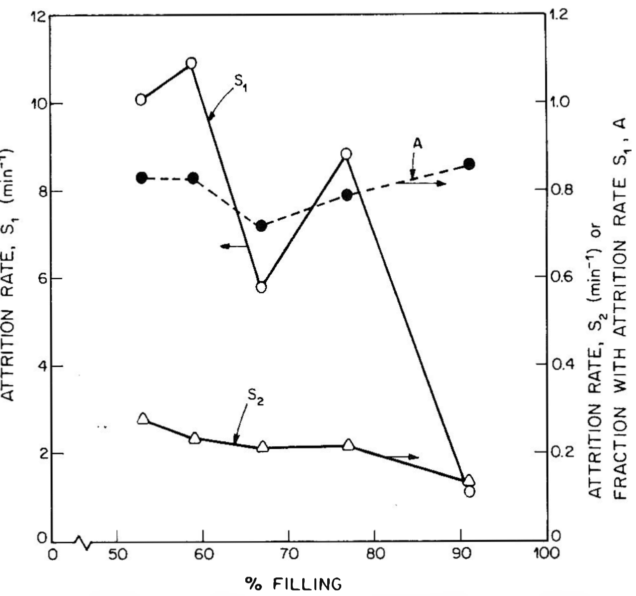

Fig. 10—Effect of the filling ratio on the attrition parameters of agglomerated instant coffee determined by a three parameter exponential model (Fig. 2). S_1 and S_2 are rate characteristics and A the fraction with an attrition rate S_1. (−6+8 mesh fraction in vertical vibration at 300 rpm).

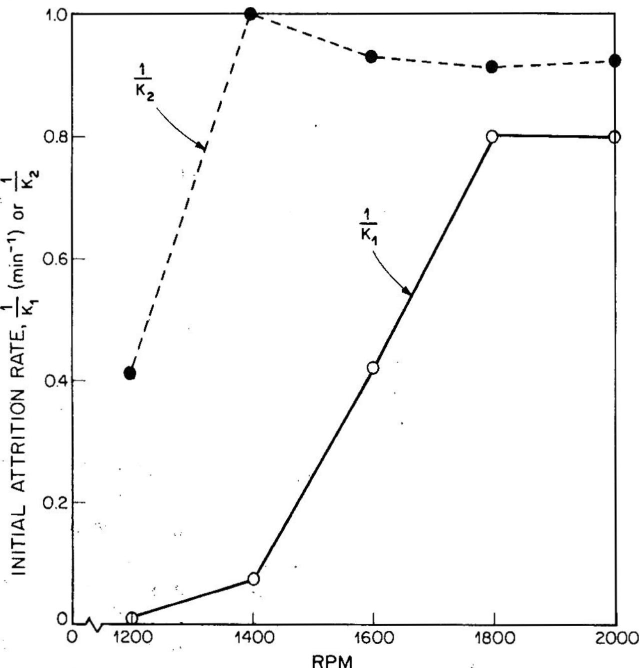

Fig. 9—Effect of horizontal vibration frequency on the initial attrition rate ($1/K_1$) and the asymptotic fraction of attrited material ($1/K_2$), of −6+8 mesh instant coffee agglomerates. (K_1 and K_2 were determined from the two parameter nonexponential model shown in Fig. 2).

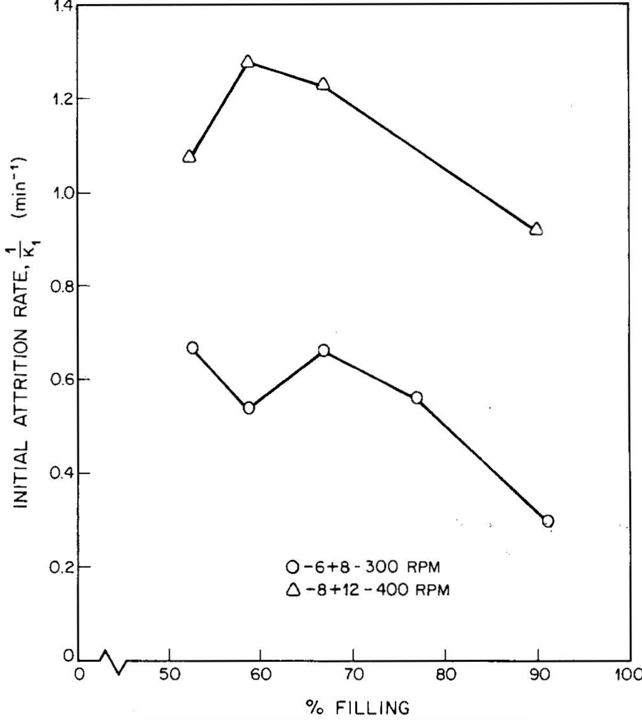

Fig. 11—Effect of filling ratio on the initial attrition rate ($1/K_1$) of agglomerated instant coffee determined by a two parameter nonexponential model (Fig. 2). Vertical vibration.

intensities were sufficient to overcome the more stable structure of the smaller agglomerates.

CONCLUSIONS

THE THREE PARAMETER exponential and the two parameter nonexponential models that were applied to experimental attrition data yielded a similar picture as to the progress of the attrition process and the role of the various factors that affect its intensity. As could be expected, the agglomerate size, the vibration frequency and the filling ratio had a considerable effect on the attrition rate and on the survivability of the original agglomerates. Their exact role, however, could only be established in an empirical way. This was primarily for the reason that the powder's mechanical histories were not clearly defined in terms of basic units. This does not mean that calculation of vibration intensity in terms of mean momentum or energy dissipation rate, for example, will resolve the issue, because relating such parameters to events occurring at the particle level, particularly with an agglomerate shape, is still an unsolved problem.

What is important, however, is the fact that despite the com-

—*Continued on page 697*

Patterns of Size Distribution Changes During the Attrition of Instant Coffees

J. MALAVE-LOPEZ and M. PELEG

ABSTRACT

Instant agglomerated and freeze-dried coffee particles of uniform size were subjected to mechanical attrition through horizontal and vertical vibration, and drop tests. The particle size distributions that resulted and their change with the time in vibration or number of drops were recorded. The results are interpreted in light of the relative roles of two breakage mechanisms, namely fines generation through surface erosion, and particle shattering that produces remnants of a much larger size.

INTRODUCTION

THE BRITTLENESS and irregular shape of instant coffee particles make them susceptible to breakage and disintegration upon mechanical impact or friction. The impact and friction can be either with equipment or package parts or walls, with other particles or with both. This mechanical attrition is a source of problems when it occurs during processing and can also affect the product appearance if it happens in the consumer package. Naturally, the intensity of the phenomenon depends on the mechanical history of the particles and their exposure to friction and impact, but it is also affected by the particles' size and shape and their material strength. Recent kinetic studies of the phenomenon in instant coffees (Malave-Lopez et al., 1985a) revealed that, unlike in many common grinding processes, the size reduction cannot be represented by a first order kinetic model, particularly when large particles are involved. It was speculated, on the basis of known grinding and attrition theories (e.g. Gwyn 1965; Wei et al., 1977; Krog, 1980; Khoe et al., 1984) that this was a result of the existence of two breakage mechanisms, one involving the shattering of the particle to product subunits of a substantial size and the other that produces fines primarily through surface erosion (Fig. 1). In that first study, the attrition progress was monitored by following the decrease of the weight fraction of the particles with the original size, a method widely used in the evaluation of size reduction operations (e.g. Mika et al., 1967; Austin, 1972; Austin et al., 1981). Because this method is based on monitoring the behavior of a single size fraction, it was difficult to substantiate the two mechanism hypotheses for lack of sufficient evidence. Support for such a hypothesis was produced, however, by testing coffees with narrow but different initial particle size. These experiments demonstrated that if the initial particle size were small enough the attrition proceeded in a manner that could be described by a first order kinetic model. This could be interpreted, although not conclusively, as evidence for a single dominant attrition mechanism.

Since shattering and surface erosion are at least, to some extent, stochastic processes, in the sense that the remnants of each broken particle can be subjected to the same processes that affected the parent particle, the best way to verify or refute the existence of two attrition mechanisms is to monitor the changes in the entire particle size distribution and not only

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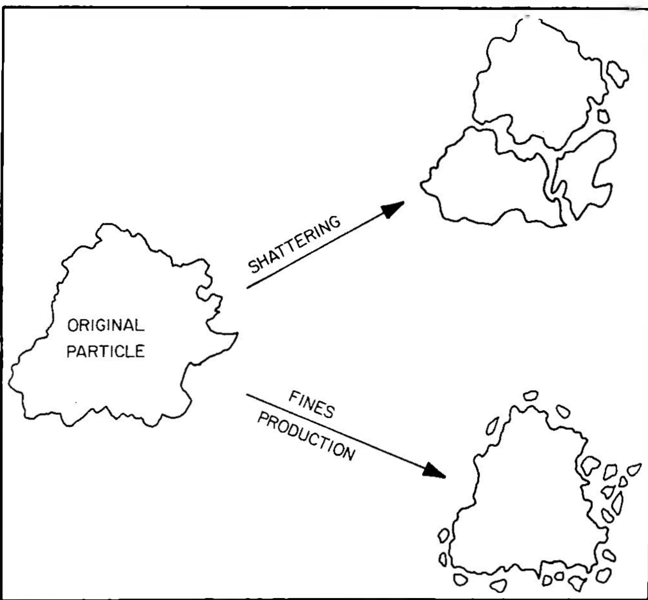


Fig. 1—Schematic view of the two main attrition mechanisms; fines production through disintegration of the surface and shattering of the particles main structure.

those that occur in the original particles. The purpose of this work was to test the applicability of this approach and to establish the attrition mechanism of commercial agglomerated and freeze dried instant coffees when subjected to mechanical vibration.

MATERIALS & METHODS

COMMERCIAL AGGLOMERATED and freeze-dried instant coffees were gently sieved to obtain fractions with narrow size distribution of −4+6 mesh (4.75–3.35mm) and −6+8 mesh (3.35–2.36mm). Samples of these fractions were placed in a metal cell and subjected to horizontal and vertical vibration under conditions previously reported by Barbosa Canovas et al. (1985) and Malave et al. (1985b). These included vertical vibration in a specially built reciprocating device operated at 400 rpm and 5 cm stroke, and horizontal vibration in a variable speed laboratory shaker (Ika-Vibrax model VXR1, Tekmar Co., Cincinnati, OH).

The particle size distribution, after various times in vibration, was determined using gentle sieving by hand (to minimize unintentional attrition). A similar procedure was applied to coffee samples that were allowed to drop repeatedly from different controlled heights. In this case, however, the size distribution is reported as a function of the number of drops (tappings) and not time as in the vibration experiments.

All the experiments were repeated with freshly prepared samples.

RESULTS & DISCUSSION

THEORETICALLY, the dominance of one of the two main attrition mechanisms, i.e. shattering and fines production (Fig. 1), could be established from the shape of the particle size distribution and its progressive change during the process. Schematic presentation of the characteristic shape of the distribution curves is shown in Fig. 2. It is possible, however,

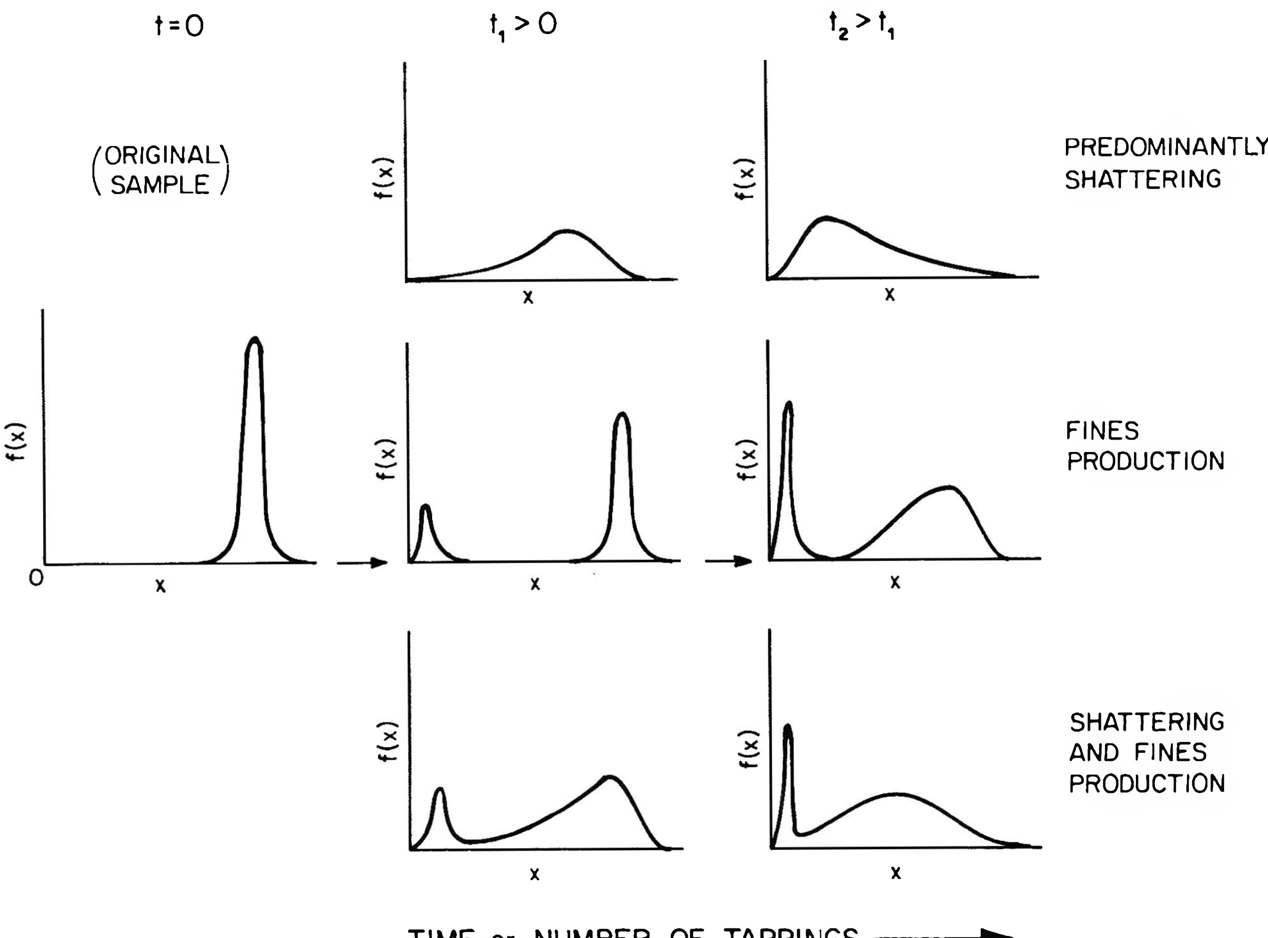


Fig. 2—Schematic view of the expected size distributions of an attrition powder when different mechanisms assume a dominant role. [f(x) is the frequence function and x the particle size, both not drawn to scale].

that the two mechanisms play a comparable role and this will be evident from a third type of a characteristic size distribution also shown in the figure. There exists, of course, the possibilities that one mechanism being dominant initially will be taken over by the other or that the dominant role will alternate between the two mechanisms. Although such situations cannot be ruled out, they seem unlikely if the mechanical conditions to which the powder is subjected remain unchanged or if the process duration is not extremely long.

Horizontal and vertical vibration

The changes in the size distribution of agglomerated instant coffees subjected to horizontal and vertical vibration are shown in Fig. 3 and 4. These figures demonstrate that the disappearance of the original particles corresponded to the creation and progressive increase in a fraction of fine particles with a mean diameter of about 0.25 mm. The same fine fraction appeared in both modes of vibration which strongly suggests that these fines were the result of surface abrasion rather than the remnants of particles disintegration by shattering. The disappearance of the original agglomerates was also accompanied by the creation of intermediate size fractions with particle size on the order of 1–2 mm. This fraction, however, reached significant proportions only after the attrition process was in progress for some time (i.e. 1–4 min), and never outweighed the fines fraction. This observation is consistent with the hypothesis that fines production was the dominant mechanism and that the origin of the intermediate fraction is original agglomerates whose external layer was turned into fines through abrasion. The fact that a considerable amount of large agglomerates (i.e. 2 mm and above) survived for most of the process, only strengthen this view. All this does not exclude the possibility that some agglomerates were indeed shattered in the process but it does indicate that such events contributed relatively little to the overall reduction in the agglomerates size. Freeze-dried coffee particles under the same conditions showed a similar disintegration pattern as is evident from Fig. 5 and 6. The latter even more clearly shows that intermediate size fractions were late to appear and a size fraction of 1–2 mm hardly appeared at all even after about 20 min of vibration. The point made earlier, that the larger intermediate fraction is the result of abrasion, is especially evident in Fig. 6 where larger particles ($-4+6$ mesh) were subjected to attrition. It would be extremely improbable that such particles would shatter in a way that will produce very large remnants (3 mm in size) and fines only with practically no remnants in the size range 0.5–2 mm.

The rate at which the fines fraction increased depended on the mechanical conditions. It was generally higher, however, in the powders subjected to vertical vibration as shown in Fig. 7 and 8, most probably as a result of the more intense interparticle interactions under these circumstances.

Drop tests

The behavior of agglomerated coffee in drop tests is illustrated in Fig. 9. Although fines production is still prominent, there is a clear shift towards the production of an intermediate size fraction (1–2 mm) fairly early in the process. The difference, however, is only quantitative and indicates that while abrasion was still the dominant attrition mechanism, shattering has also assumed a significant role. This was more clearly evident in the case of the freeze-dried coffee as seen from Fig. 10 and 11. Comparison of these figures to Fig. 5 and 6 clearly show

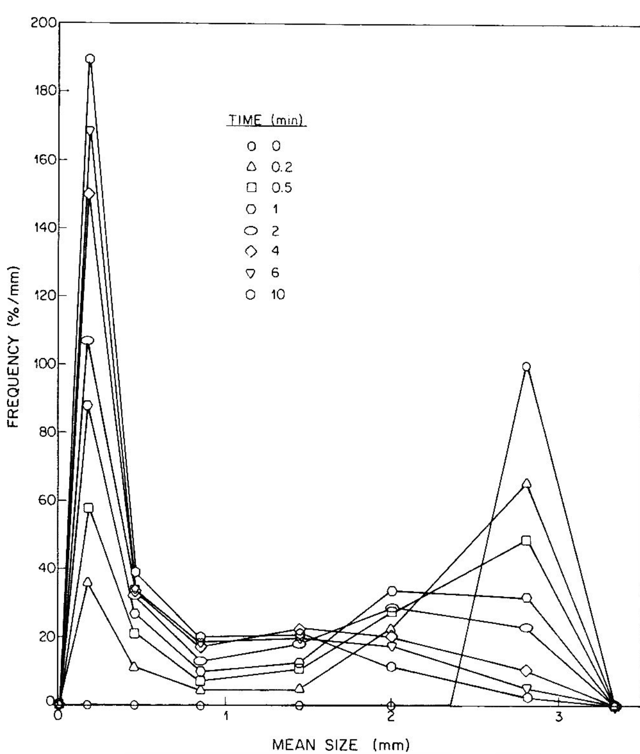


Fig. 3—Particle size distributions of −6+8 mesh fraction of agglomerated instant coffee subjected to vertical vibrations (speed 400 rpm).

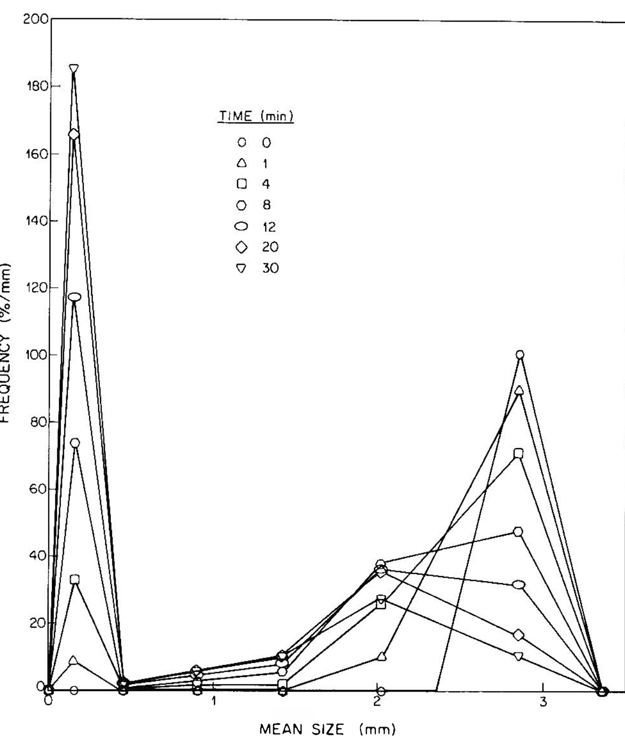


Fig. 5—Particle size distributions of −6+8 mesh fraction of freeze dried instant coffee subjected to vertical vibrations (speed 400 rpm).

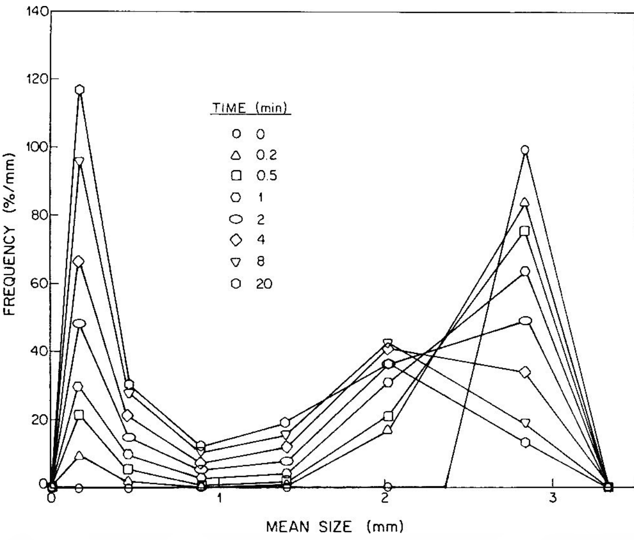


Fig. 4—Particle size distributions of −6+8 mesh fraction of agglomerated instant coffee subjected to horizontal vibrations (speed 1800 rpm).

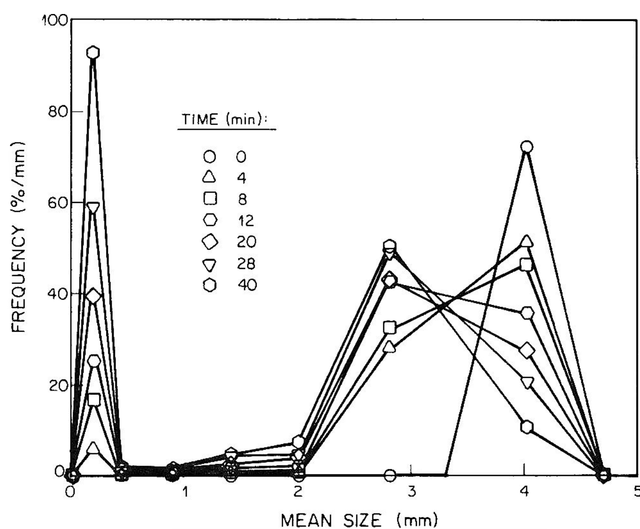
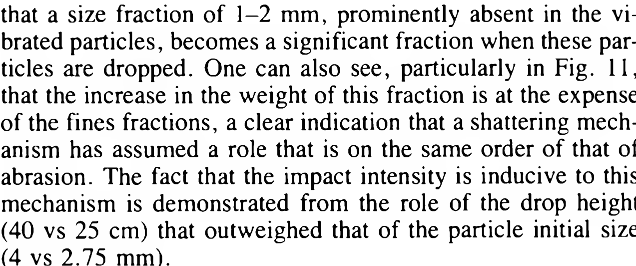


Fig. 6—Particle size distributions of −4+6 mesh freeze dried instant coffee subjected to horizontal vibrations (speed 1800 rpm).


that a size fraction of 1–2 mm, prominently absent in the vibrated particles, becomes a significant fraction when these particles are dropped. One can also see, particularly in Fig. 11, that the increase in the weight of this fraction is at the expense of the fines fractions, a clear indication that a shattering mechanism has assumed a role that is on the same order of that of abrasion. The fact that the impact intensity is inducive to this mechanism is demonstrated from the role of the drop height (40 vs 25 cm) that outweighed that of the particle initial size (4 vs 2.75 mm).

CONCLUSIONS

IT HAS BEEN DEMONSTRATED that, under the conditions of relatively "mild" impact and friction, the attrition of instant coffees, agglomerated and freeze-dried, is predominantly a process of fines production, i.e. a phenomenon that is associated with the disintegration of the external parts of the particles. The rate of the fines production depends on the intensity of the mechanical energy dissipation and is strongly affected by the system geometry. "Large" impacts, in these experiments produced by drop tests, also resulted in the shattering of particles but this was mainly a characteristic of freeze-dried coffee. These observations suggest that the structure of agglomerated coffee is such that it tends to shed small aggregates of particles which are loosely attached to the agglomerate's main body. The effect of impact as well as frictional forces will, therefore, be mainly expressed in the production of fines. Since freeze-dried coffee particles have more structural integ-

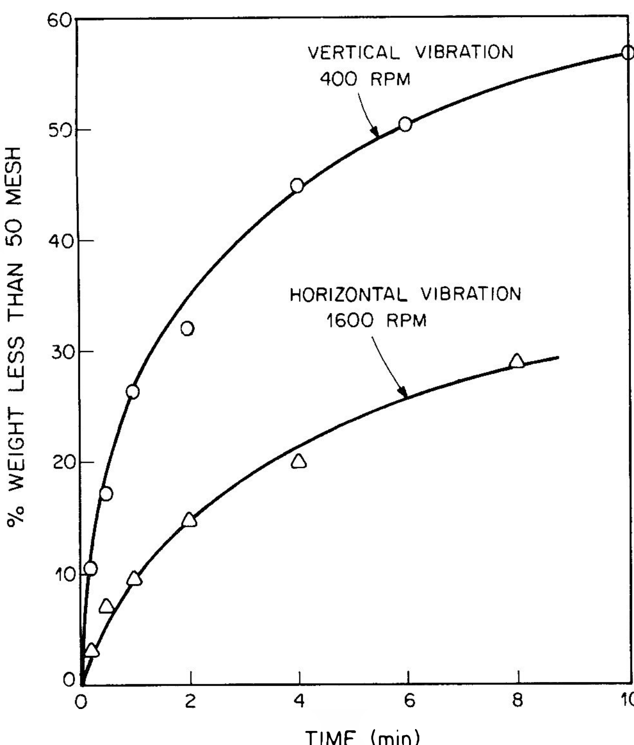


Fig. 7—Comparison of the fines production rates of agglomerated instant coffee subjected to horizontal and vertical vibration.

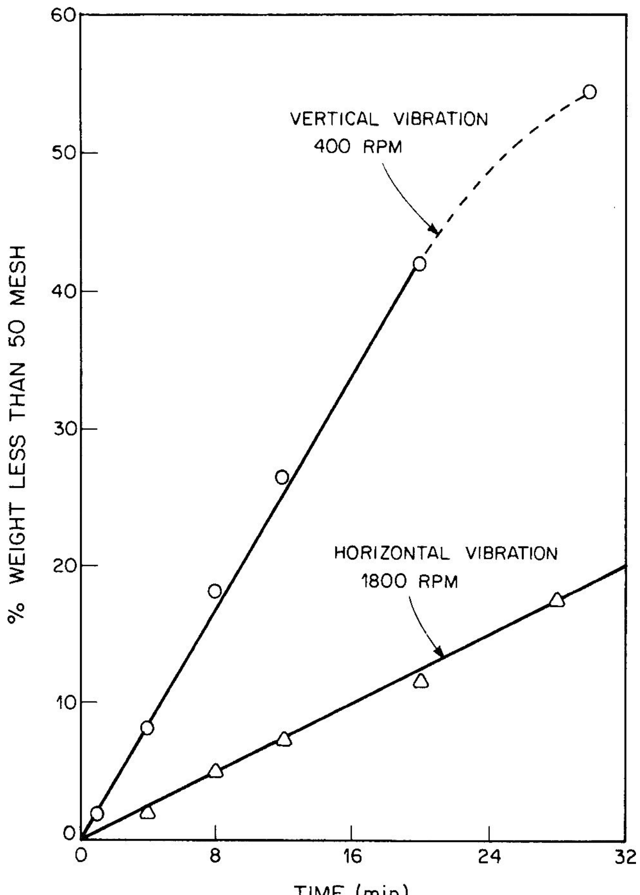


Fig. 8—Comparison of the fines production rates of freeze dried instant coffee subjected to horizontal and vertical vibration.

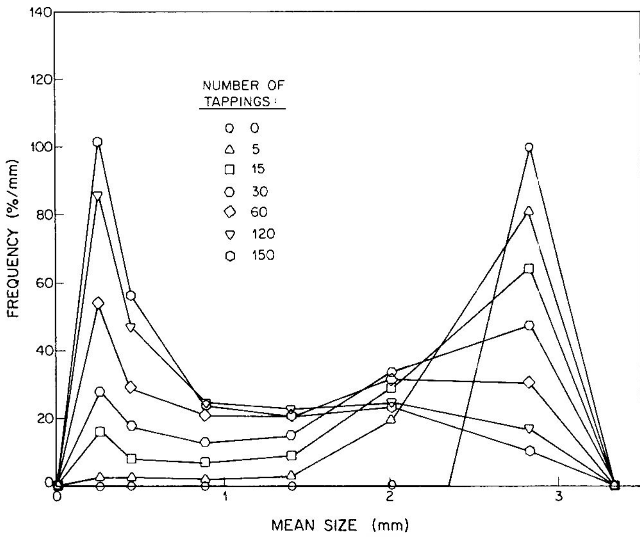


Fig. 9—Particle size distributions of −6+8 mesh fraction of agglomerated instant coffee subjected to tapping (drop height 10 cm).

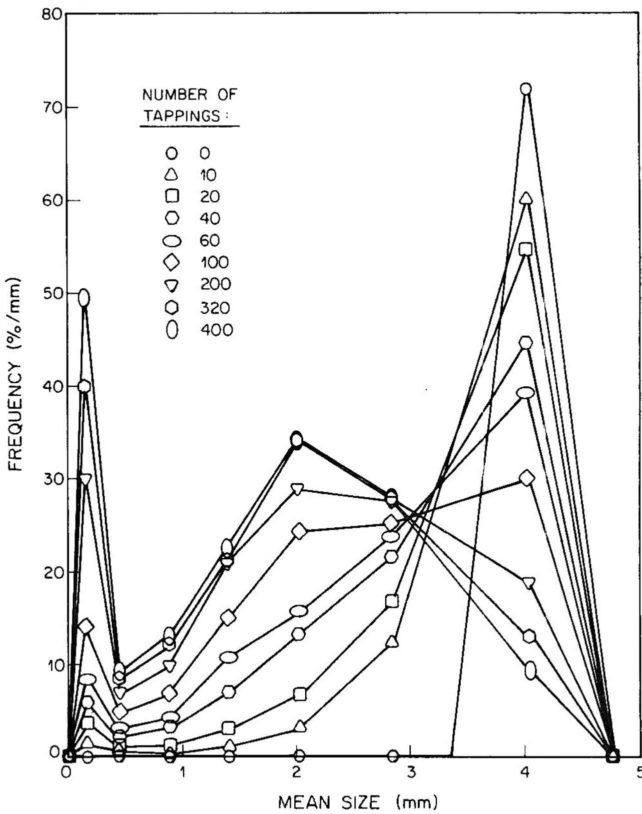


Fig. 10—Particle size distributions of −4+6 mesh fraction of freeze dried coffee subjected to tapping (drop height 25 cm).

rity, i.e. despite being porous, they are not "branched," there is a noticeable difference in the effects of friction and impact on their size reduction. While the former is mainly in the form of fines production and rounding of the surface, the latter causes shattering of the particles to produce large remnants as well as a certain amount of fines.

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Coconut Oil Extraction by a New Enzymatic Process

OCTAVIO CINTRA McGLONE, AGUSTÍN LÓPEZ-MUNGUIA CANALES, and JAIME VERNON CARTER

ABSTRACT

A new method for coconut oil extraction based on the enzymatic action of polygalacturonases, α-amylase and proteases on a diluted coconut paste was developed. After reaction with enzymes, the solution was centrifuged resulting in three phases: the upper one containing a high quality coconut oil; the middle layer containing water; and the lower phase consisting of coconut meal which has been traditionally used in cattle feed. Eighty percent yields were obtained by this method which was advantageous from the energy point of view compared to the traditional process.

INTRODUCTION

CURRENTLY, two main processes for the extraction of oil from seeds are of industrial importance: the hydraulic process plus further purification, and the chemical process, using organic solvents (Hagenmainer et al., 1973; Woodroot, 1979). An important raw material for oil production is the coconut, which provides the food and other industries with an oil of excellent quality. Nevertheless, the actual process results in at least 30% losses both in the process and in the treatment of the raw materials.

Oil is usually inside of vegetative cells, linked with other macromolecules, so that upon partial hydrolysis, oil extraction can be enhanced (Gunetileke and Laurentius, 1974). Since these macromolecules may include proteins and a wide variety of carbohydrates (starch, cellulose, hemicellulose, pectin), the hydrolysis treatment should be carried out by means of appropriate enzymes. The objective of this work was to develop a method for coconut oil extraction based on a biological process in order to minimize the energy cost of extraction. There are no reports in the literature specifically related with such a process, although enzymes have been used to enhance extraction in industrial processing of some plant tissues (Godfrey and Reichelt, 1983).

MATERIALS AND METHODS

COCONUTS, obtained in Guerrero, México (Jamaica tall), were classified in terms of maturity by proximate analysis. Polygalacturonases (Clarex), amylases (Tanase), cellulases (Celluferm), and proteases (HT-proteolytic) were kindly provided by ENMEX, S.A.

Proximate analysis

Fat was measured by the Soxhlet method, protein by Kjeldahl and fiber, ash and carbohydrates as reported in AOAC (1975).

Emulsion stability

Emulsion stability was determined by the method of Sherman (1968), once the optimal dilution for enzymatic reaction was found. The method is based on the measurement of particle size in a drop of a solution prepared with the emulsion. This procedure was carried out as a function of enzymatic reaction time. The measurement was made with an optical standard microscope equipped with a micrometer. The average diameter (Da) was calculated as:

$$Da = \left[\frac{n_1D^3_1 + n_2D^3_2 + n_3D^3_3 + \ldots + N_iD^3_i}{n_1 + n_2 + n_3 \ldots + n_i}\right]^{1/3}$$

where $n_1, n_2, n_3 \ldots n_i$ are the numbers of particles or oil drops with diameter $D_1, D_2, D_3 \ldots D_i$ respectively.

In order to evaluate the stability, the number of particles by unit volume (N) for each diameter was determined using the following equation:

$$N = \frac{6\,\phi \times 10^{12}}{\pi\, Da^3}$$

where ϕ is the volume fraction of the disperse phase, defined as:

$$\phi = \frac{\text{Volumetric conc of disperse phase}}{\text{Volumetric concentration of disperse phase} + \text{Volumetric concentration of continuous phase}}$$

With these data a graph of log N versus time (in which the aliquots were taken) can be constructed. A straight line is obtained; the slope is the coalescence rate. The stability of the emulsion is inversely proportional to the coalescence rate; the emulsion is stable if this parameter falls between 10^{-7} to 10^{-12}, but it is not stable if higher values are obtained.

Enzymatic and extraction procedures

Before the addition of enzymes and dependent on the hydrolytic nature of the reactions involved, a proper dilution of the chopped

Table 1—Proximate analysis of four different samples of coconut meat

Constituent	g/100g coconut meat	Standard deviation
Water	48.12	1.95
Protein	3.8	0.15
Oil	27.6	0.4
Ash	0.91	0.03
Crude fiber	16.9	0.65
Carbohydrates[a]	2.67	—

[a] By difference.

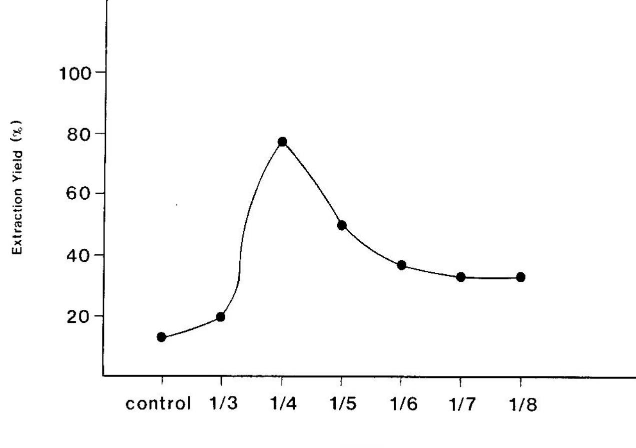

Fig. 1—Effect of coconut meat dilution on oil extraction yield. (Enzymes 1%, T=40°C). After reaction, the emulsion was centrifuged at 12,300 x g).

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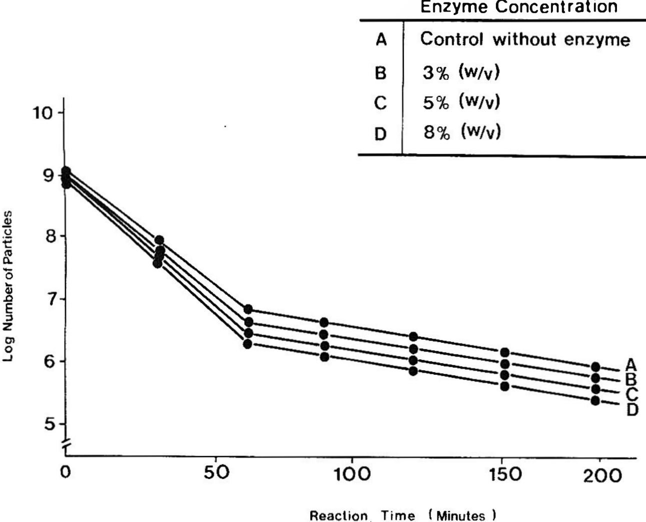

Fig. 2—Emulsion stability during the enzymatic reaction at 40°C at different enzyme concentrations (N = number of particles per unit volume).

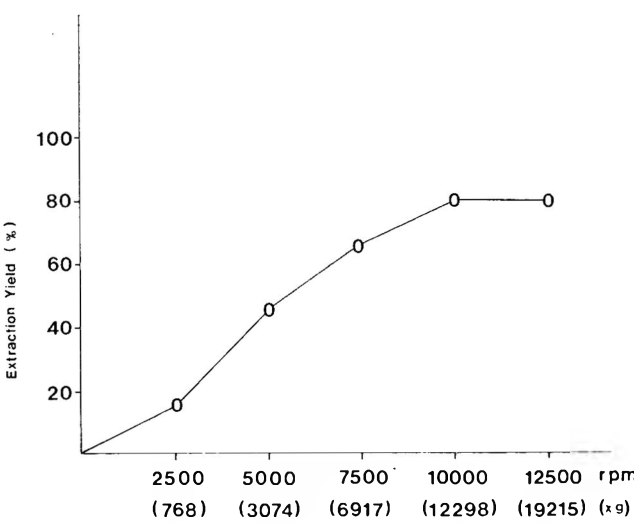

Fig. 4—Effect of centrifugation speed on extraction yield after enzymatic reaction (centrifugation time: 10 min).

Table 2—Selection of enzymes for the oil extraction process in terms of extraction yield

Enzymes[a]	Extraction yield %
Control	12
Protease	19
α-Amylase	31.2
Polygalacturonase (PG)	40.1
PG and protease	49
PG and α-amylase	58.9
PG, α-amylase and protease	80

[a] All enzymes added at 1% w/v.

Table 3—Selection of enzyme concentration in the extraction process in terms of extraction yield

Enzyme concentration (% w/v)			Extraction yield (%)
PG[a]	α-Amylase	Protease	
1	1	1	80
0.5	0.5	0.5	80
0.1	0.1	0.1	80
0.01	0.01	0.01	49
0.1	0.05	0.05	65

[a] Polygalacturonase

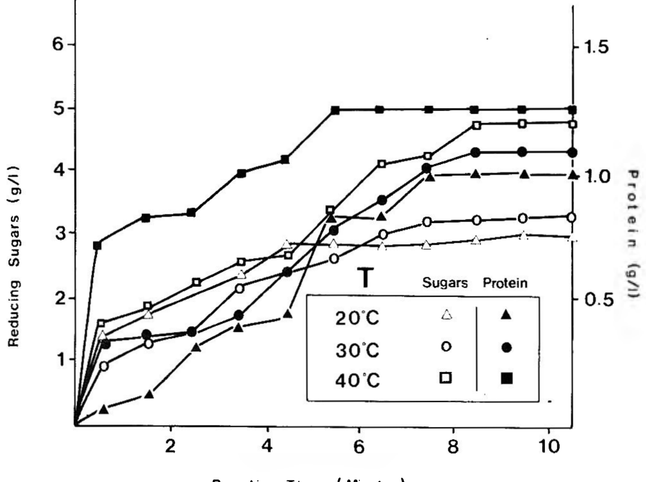

Fig. 3—Effect of time and temperature on enzymatic reactions of coconut meat solution (1:4), determined as reducing sugars (glucose) and soluble protein (albumin). A mixture of 0.1% (w/v) of all the enzymes was used.

Table 4—Characterization of the extracted oil

	Extracted Oil	Official Mexican Standard[a] Minimum	Maximum
Free fatty acids (as oleic) %	0.07	—	0.05
Specific gravity at 25/25°C	0.92	0.917	0.919
Refractive index	1.450	1.448	1.450
Iodine absorption number	9.0	7.5	—
Saponification number	259	251	264
Melting point of fats and fatty acids (°C)	23	20	24
Peroxide value (ppm)	0.90	—	2.0
Reichter-Meissl value %	0.05	—	0.05

[a] SECOFI (1976)

(Moulinex grinder) coconut meat was investigated, forming an emulsion at the same time. To evaluate the optimal dilution level, a mixture of enzymes (amylases, polygalacturonases (PG), proteases and cellulases) in dry form was added to the emulsion prepared at different coconut meat/water ratios. After 30 min reaction at 40°C, the solution was decanted and centrifuged at 12,300 x *g* and the extracted oil quantified.

The centrifugation conditions were also optimized. The reaction product was centrifuged (DAMON/IEC Division) in the range of 760–19,200 × *g* at room temperature (20–22°C) and the extraction yield quantified.

A semipilot plant experiment was carried out in a 14L fermentor used as a reactor vessel. Two kilograms of coconut meat were chopped and mixed with water in a 1:4 ratio. The temperature was fixed at 40°C, 0.1% of enzymes added (w/v) and agitation was provided for the enzymatic reaction (200 rpm). After 20 min of reaction the emulsion was decanted and centrifuged at 12,300 x *g*.

Reduced sugars and proteins were determined by the methods of Summer and Howell (1935) and Lowry et al. (1951), respectively. Finally, the quality of the product was evaluated according to the Official Mexican standard (SECOFI, 1976), following the methods reported in AOAC (1975).

Extraction yield

The reported yield was calculated based on the initial coconut oil content (determined by Soxhlet) and direct measurement of the volume and weight of the oil obtained after centrifugation of the reaction mixture.

RESULTS & DISCUSSION

THE RESULTS of the proximate analysis of four different samples of coconut meat are shown in Table 1. The selected samples were in a similar state of maturity and served as the basis for evaluating the efficiency of the extraction process as well as the selection of enzymes that could react with the coconut tissue.

A dilution of 1:4 gives the highest extraction yield (Fig. 1). At this dilution level, the stability of the emulsion was determined at three different enzyme concentrations as described. These results are summarized in Fig. 2, from which it can be concluded that the emulsion formed is very unstable and rapid separation of oil can be achieved. This instability was not related to the action of enzymes as the coalescence rates were very small (slope of the straight lines) and there were no significant differences between the control and the emulsions with the added enzymes.

In order to select the enzymes that were involved in the separation process, the reaction was carried out using each one separately as well as mixtures, all being added at 1%. The results are shown in Table 2, where the maximum extraction yield was found for the mixture of the three enzymes, after eliminating the cellulase because of the lower activity with the emulsion. This result was probably due to the low cellulose content in the cellular structure from which oil was liberated.

Once these three enzymes were selected, the concentration was optimized. The same extraction yield was obtained even when the concentration was reduced tenfold from the original, which was selected in terms of activity units as defined by the manufacturer. These results are shown in Table 2.

To characterize the modification carried out by the enzymes during the reaction, both reducing sugars and soluble protein were measured during the reaction at three different temperatures. These results are reported in Fig. 3 where no further change is observed after 10 min, both in reducing sugars and soluble protein. The highest yield after centrifugation was obtained for the reaction carried out at 40°C; therefore, time of reaction and temperature were defined with the experiment.

The final step of the extraction was centrifugation which could be carried out at different conditions. When the centrifugation time was fixed at 10 min, the extraction yield increased with centrifugation speed until a maximum of 80% was obtained at 12,300 × *g*. These results are shown in Fig. 4.

In the semipilot plant experiment, a final volume of 420 mL was obtained from 2 kg of coconut meat. This volume corresponded to a 74.7% yield which was slightly lower than the 80% obtained in the laboratory experiments. This yield reduction was certainly due to the particle size which played an important role in the extraction process. Particles could not be reduced in the semipilot plant experiment to the small size obtained in the laboratory.

Results of the quality of the oil are reported in Table 3 where the analyses of the product are compared with the Official Mexican standard. Without any further purification, the oil, obtained after reaction and centrifugation, had an excellent quality and with a simple deodorization process could be readily used in existing food applications.

CONCLUSIONS

COCONUT OIL in high yields and good quality compared to Official Mexican standards can be produced by a new process which utilizes enzymes to hydrolyze cellular material in coconut meat and release the oil which can be recovered by centrifugation. Similar processes for the extraction of oils from other seeds should be feasible and are now being investigated.

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ATTRITION OF COFFEE. . .From page 690

plexity of the attrition phenomenon and its dependency on some difficult to define or measure factors (e.g. agglomerate morphology and strength, interparticle impact intensity, accelerations and their distribution in the bed, and the temporal relationships of all the above), the process itself can still be described by simple phenomenological models which were found to be applicable under a wide range of experimental conditions. The mathematical methods described in the work, despite being unspecific from a mechanistic viewpoint and, to some extent even crude, were still sensitive enough to capture the main features of the attrition kinetics, thus making them practical and convenient tools for the phenomenon's analysis and quantitative evaluation of its progress.

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Degradation of *trans*-β-Carotene during Heating in Sealed Glass Tubes and Extrusion Cooking

C. MARTY and C. BERSET

ABSTRACT

Four compounds which have been previously reported as products of enzymatic and chemical oxidation reactions were isolated and identified for the first time from the epoxide fraction formed by heating all *trans*-β-carotene. A HPLC method allowing a quick separation and identification of these epoxides was developed. Extrusion cooking at 180°C was a more drastic treatment for the pigment than simple heating for a long period (2 hr) at the same temperature.

INTRODUCTION

CAROTENOID PIGMENTS possess a polyene structure which gives them considerable reactivity towards light and heat. β-Carotene, a vitamin A precursor, widely present in nature, is chemically synthesized for use as a food coloring. Hence its thermal degradation is of concern to food manufacturers and nutritionists.

Among the thermal degradation compounds of β-carotene, volatile products have been extensively studied and identified by gas chromatography/mass spectrometry. Many investigations have reported the formation of toluene, m- or p-xylene, 2,6-dimethylnaphthalene and ionene (Day and Erdman, 1963; Mader, 1964; Ikan et al., 1975; Hinnekens et al., 1976; Schrier et al., 1979; Ishiwatari, 1980). Heating β-carotene in the presence of air, Schrier et al. (1979) also found oxidized volatile compounds such as β-cyclocitral, β-ionone, 4,6-epoxy-β-ionone, 4-oxo-β-ionone and dihydroactinidiolide.

High molecular weight compounds are much less known and their identification is sometimes doubtful. The presence of many degradation compounds, in small amounts, with similar polarities and high reactivity necessitates sensitive purification and identification methods. On account of the high instability of the purified products, only methods which avoid degradation reactions during analysis can be used. Moreover, the small amount of each compound available requires methods consuming small quantities, such as mass spectrometry. However, this method is not always sufficient to establish the structure accurately.

All these reasons explain the small amount of systematic research carried out and the incomplete literature data on this subject. Zinsou (1973) isolated some β-carotene auto-oxidation compounds with epoxide, carbonyl and hydroxide functions and characterized them by UV-visible spectrophotometry and chemical reactions. Ouyang et al. (1980) identified, with infrared spectrophotometry and electron-impact mass spectrometry, three oxidized thermal degradation compounds: two apo-carotenals and one apo-carotenone. Onyewu et al. (1982) identified, with the same spectroscopic methods, two polyenic thermal degradation compounds of molecular formula $C_{29}H_{42}$ and $C_{33}H_{48}$. However, many β-carotene degradation products remain unidentified today.

β-Carotene is widely used in the food industry and is often subjected to high temperatures, especially for confectionery products. On the other hand, extrusion cooking is becoming more widespread in the food industry, but only two papers have reported on carotenoid stability during the extrusion process. Lee et al. (1978) indicated that β-carotene and canthaxanthin were relatively unstable in extrusion cooking. Likewise, Kone and Berset (1982) reported that commercial β-carotene powders were subject to structural change during residence time in the extruder. Thus, it seems important to identify the compounds arising from β-carotene degradation in the extruder, which can lose their provitamin A characteristics or exhibit possible toxicity.

The objective of this study was to carry out the structural analysis of the β-carotene epoxide derivatives, using chemical-ionization mass spectrometry and other spectroscopic methods and to establish a reproducible fractionation method by high performance liquid chromatography for these compounds in order to compare the results of all *trans*-β-carotene degradation during two different thermal processes : heating in sealed glass tubes and extrusion cooking.

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Table 1—Fragmentations of DP_1 in electron-impact mass spectrometry

m/e	Fragmentation	Resultant ion
536	loss of oxygen M - 16 (O)	$C_{40}H_{56}^{7+}$ very weak peak
472	M - 80 (C_6H_8)	$C_{34}H_{48}O^{7+}$
406	M - 146 ($C_{11}H_{14}$)	$C_{29}H_{42}O^{7+}$
368	M - 184 ($C_{14}H_{16}$)	$C_{26}H_{40}O^{7+}$
336	M - 216 ($C_{16}H_{24}$)	$C_{24}H_{32}O^{7+}$
205		$C_{14}H_{21}O^{7+}$
91		$C_7H_7^{7+}$

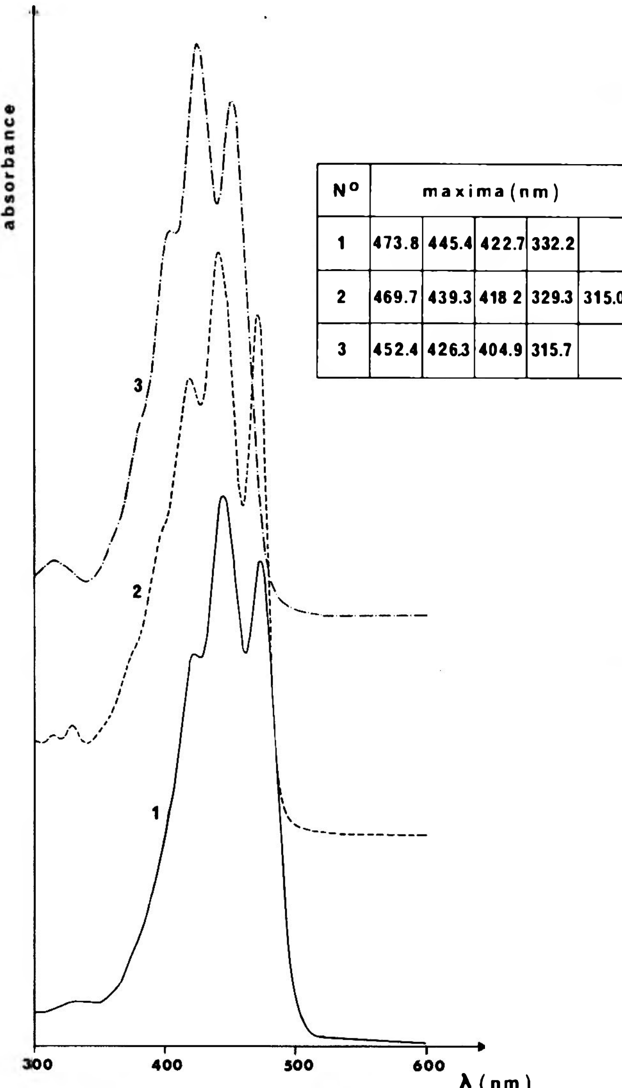
MATERIALS AND METHODS

SOLVENTS were distilled and tested by gas chromatography before use. All chromatographic runs were performed at 4°C in darkness to avoid thermal and photochemical reactions during the fractionation process.

All trans-β-carotene purification

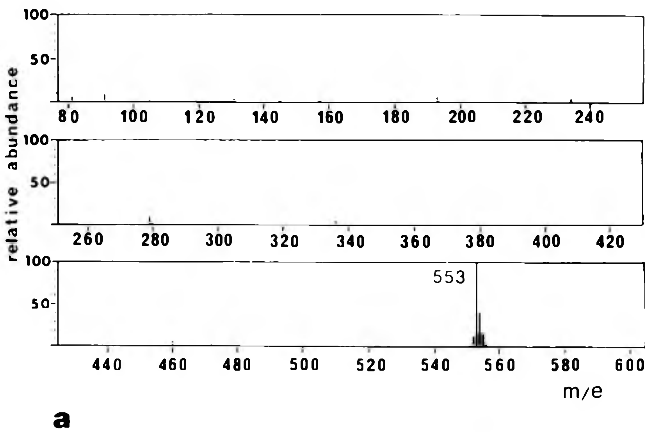
Synthetic β-carotene (Fluka purum grade) was successively purified on two alumina columns (alumina II-III and alumina I, Merck distributed by Socolab Paris France, granulometry: 0.063–0.200 mm). Alumina II-III was packed into a 3 cm i.d. column, to a depth of 20 cm. One hundred milligrams of β-carotene were mixed with 400 mg of alumina and added to the top of the column. The first fraction (A) containing all *trans*-β-carotene and *cis* isomers, was eluted with 10% diethyl ether in n-hexane. This fraction was further separated into two fractions, A_1 and A_2, using a similar column packed with alumina I and a stepwise gradient elution, 20% diethyl ether in n-hexane (A_1) and 30% diethyl ether in n-hexane (A_2).

The fraction, A_2, containing all-*trans*-β-carotene was collected and the pigment was crystallized from methylene chloride. The purity was tested by melting point determination, UV-visible, infrared, electronic impact mass spectrometry (EIMS), chemical ionization mass spectrometry (CIMS), proton nuclear magnetic resonance (1H NMR) and



N°	maxima (nm)				
1	473.8	445.4	422.7	332.2	
2	469.7	439.3	418 2	329.3	315.0
3	452.4	426.3	404.9	315.7	

Fig. 1—UV-visible spectra of DP_1 (1), DP_2 (2) and DP_3 (3) in n hexane.



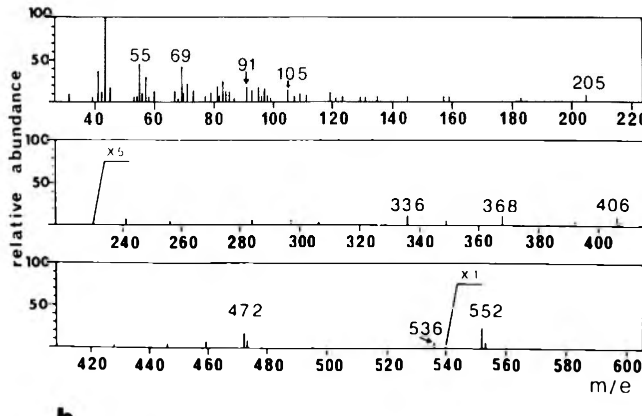


Fig. 2—Chemical ionization/desorption mass spectrum (a) and electron-impact mass spectrum (b) of DP_1.

carbon nuclear magnetic resonance (13C NMR) spectra (Marty and Berset, 1986).

Heating in sealed glass tubes

A methylene chloride solution of the pure all-trans pigment was introduced into glass tubes (50 mg per tube). To obtain a homogeneous distribution of the pigment on the walls, the solvent was evaporated while the tube was maintained in a horizontal position while being rotated. The glass tubes were sealed and heated in an oil bath for 2 hr at 180°C, then cooled and kept at −30°C until further use.

Extrusion cooking

Four hundred milligrams of the purified all trans-β-carotene dissolved in 250 mL of chloroform were mixed with 50g of corn starch (Roquette Frères, 62136 Lestrem, France). The mixture was stirred magnetically for 1 hr and dried, first under vacuum then under a flow of nitrogen. The starch-pigment mixture was incorporated into 5 kg of starch in a rotary mixer and mixed for 5 hr. The extrusion experiments were performed in a twin screw BC 45 extruder (Clextral, 42701 Firminy, France). The processing conditions were as follows: barrel temperature in the final zone, near the dies, was set at about 180±2°C, screw speed was 150 rpm, feeding rate was 25 kg/hr, amperage was about 23 A. A continuous flow of 2.4 L/hr water was mixed with the paste at the inlet of the screws. After reaching steady-state conditions, a 15 min running time was set up before collecting samples. A rotary cutter produced spherical extrudates which were kept in thermo-welded bags at −30°C until further use.

Pigment extraction process

To fifty grams of ground extrudates were added: 1 L of distilled water; 100 mL of hydroquinone solution in absolute ethanol (2 g/L); 250 mL of phosphate buffer (0.05M, pH 7.0); 50 mL of α-amylase aqueous solution (10 g/L).

The contents of the conical flask were stirred magnetically for 2 hr at ambient temperature in the dark to carry out enzymatic digestion of the starch. The pigments were extracted twice with 1L of hexane-acetone (60:40 v/v). The organic phase was washed twice with 500 ml of distilled water, dried with anhydrous sodium sulfate and evaporated under vacuum at 30°C. The pigments were kept at −30°C until the chromatographic separation.

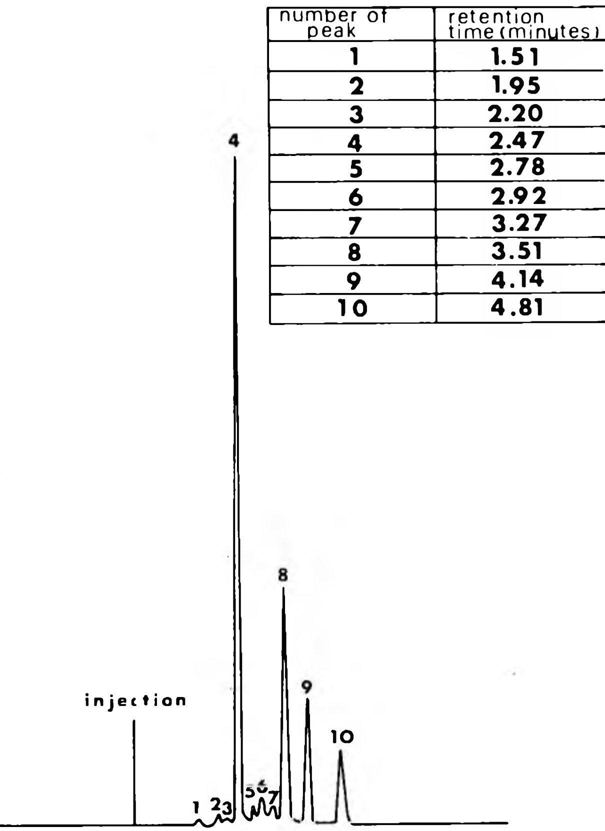
To verify the absence of degradation during the mixing with corn starch and the extraction, a sample of pigment-corn starch mixture was subjected to the same procedure and compared with the pure β-carotene.

Column chromatography

The pigment mixture obtained after heating or extrusion cooking was first fractionated on a preparative alumina column (alumina II-III Merck, granulometry: 0.063–0.200 mm). Five fractions were separated by a stepwise gradient elution: 10% diethyl ether in n-hexane (Fraction A); 30% diethyl ether in n-hexane (Fraction B); 40% diethyl ether in n-hexane (Fraction C); 100% diethyl ether (Fraction D); and pure methanol (Fraction E). To verify the absence of degradation during the chromatographic separation, pure all trans-β-carotene was also subjected to column chromatography.

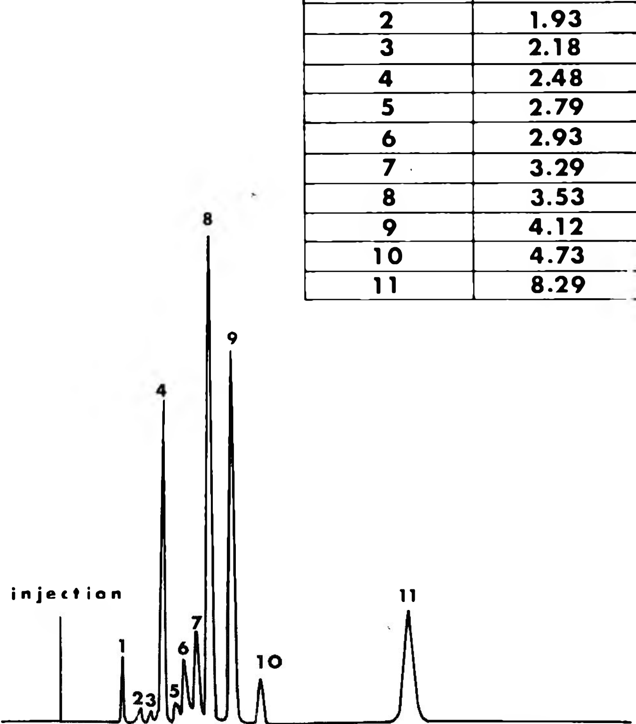
Thin-layer chromatography (TLC)

TLC experiments were performed on alumina I plates (Merck aluminium oxide 60 F_{254} neutral, layer thickness 0.2 mm) with 10% diethyl ether in n-hexane as solvent. Each colored band was recovered in methylene chloride and rechromatographed twice. After filtration



number of peak	retention time (minutes)
1	1.51
2	1.95
3	2.20
4	2.47
5	2.78
6	2.92
7	3.27
8	3.51
9	4.14
10	4.81

Fig. 3—HPLC chromatogram of β-carotene epoxides (fraction B) formed during heating in sealed glass tubes. Stationary phase-Lichrosorb SI 60; pressure-32 bars; flow rate-1.0 ml/min; temperature-ambient (22°C); detection-420 nm; eluent-5% diethyl ether in n-hexane. Peak 1- remaining all trans-β-carotene in fraction B; peaks 2,3-cis isomers of β-carotene-5,6-epoxide; peak 4-all trans-β-carotene-5,6-epoxide; peaks 5,6,7,9-cis isomers of β-carotene-5,8-epoxide; peak 8-all trans-β-carotene-5,8-epoxide; peak 10- all trans-β-carotene-5,6,5',6'-diepoxide.



number of peak	retention time (minutes)
1	1.52
2	1.93
3	2.18
4	2.48
5	2.79
6	2.93
7	3.29
8	3.53
9	4.12
10	4.73
11	8.29

Fig. 4—HPLC chromatogram of β-carotene epoxides (fraction B) formed during extrusion cooking. Stationary phase-Lichrosorb SI 60; pressure-32 bars; flow rate-1.0 ml/min; temperature-ambient (22°C); detection-420 nm; eluent-5% diethyl ether in n-hexane. Peak 1- all trans-β-carotene; peaks 2,3-cis isomers of β-carotene-5,6-epoxide; peak 4-all trans-β-carotene-5,6-epoxide; peaks 5,6,7,9-cis isomers of β-carotene-5,8-epoxide; peak 8 -all trans-β-carotene -5,8-epoxide; peak 10-all trans-β-carotene-5,6,5',6'-diepoxide; peak 11- all trans-β-carotene-5,6,5',8'-diepoxide.

and evaporation under a flow of nitrogen, each sample was kept at −30°C.

High pressure liquid chromatography (HPLC)

HPLC fractionation of the mixture of identified pigments was carried out on a Lichrosorb SI 60 column (125 mm × 4 mm, Merck, prepacked column). Five percent diethyl ether in n-hexane was used to obtain a satisfactory separation. Detection was carried out at 420 nm on a model 8800 UV-Visible spectrophotometer (Dupont de Nemours France, BP 85, 91403 Orsay).

Spectrometry

UV-visible spectra were performed in n-hexane (Carlo Erba, 92081 La defense, France, RS grade for UV-visible spectrophotometry) on a Uvikon 810 UV-visible spectrophotometer (Kontron, 78194 Trappes, France) between 300 and 600 nm. Infrared spectra were obtained on a Perkin-Elmer IR 4000 (Perkin-Elmer SA, 78390 Bois d' Arcy, France). Purified samples were run as KBr micropellets. Mass spectra in electron-impact or chemical-ionization/desorption mode (NH_3, CH_4) were obtained on a Ribermag R10-10 mass spectrometer (Nermag, 92502 Rueil-Malmaison, France). Ionization voltage in EIMS was 70 eV and pressure in CIMS was 0.1 Torr.

Chemical reactivity of the epoxide functions

Compunds containing an epoxide function are rapidly isomerized to the corresponding furanoid oxide compounds in the presence of traces of acid. This structural change causes a hypsochromic shift of the absorption maxima in ethanol: about 20 nm for mono-epoxide compounds and about 40 nm for di-epoxide compounds (Goodwin, 1980). To test the presence of β-carotene-5,6-epoxide function(s), one drop of 0.1M HCl was added directly to the ethanolic solution of the pigment in the spectrophotometer cuvette.

Epoxidation reaction

One-tenth or two-tenths mole of metachloroperbenzoic acid (Aldrich, 67000 Strasbourg, France) was added to one mole of all trans-β-carotene in methylene chloride solution. The solution was introduced into a flask topped with a $CaCl_2$ guard and immersed in an ethanol bath at 0°C with magnetic stirring for 1 hr. The pigment mixture was separated by TLC and each band was further studied by UV-visible spectrophotometry and mass spectrometry. Spectral data were compared to those of all trans-β-carotene degradation compounds appearing during heating.

RESULTS & DISCUSSION

Identification of epoxide derivatives formed during all *trans*-β-carotene heating in sealed glass tubes

Column chromatography of all *trans*-β-carotene before heating in sealed glass tubes gave only fraction A including 98% of all *trans*-β-carotene. Alumina column chromatography did not therefore involve a degradation of the pigment.

TLC separation of the pigments contained in fraction B, after heating of all *trans*-β-carotene for 2 hr at 180°C, resulted in three colored bands of degradation products (DP) $-DP_1$, DP_2, DP_3- with R_f respectively 0.7, 0.6, and 0.5. Their infrared spectra showed a polyene structure and one or several epoxide functions and their UV-visible spectra (Fig. 1) were compared with those of carotenoids containing epoxide functions available in the literature (Davies et al., 1970; Stransky and Hager, 1970; Zinsou, 1973). According to these spectral

Fig. 5—Reactions sequence for the formation of β-carotene epoxides during heating. 1 — β-carotene; 2 — β-carotene-5,6-epoxide; 3 — β-carotene-5,8-epoxide; 4 — β-carotene-5,6,5′,6′-diepoxide; 5 — β-carotene-5,8,5′,6′-diepoxide; 6-β-carotene-5,8,5′,8′-diepoxide.

data DP_1, DP_2 and DP_3 could correspond to β-carotene-5,6-epoxide; β-carotene-5,6,5′,6′-diepoxide and β-carotene-5,8-epoxide, respectively. Such hypotheses must be confirmed by further identification methods: CIMS and EIMS.

Identification of DP_1

The chemical ionization/desorption mass spectrum of DP_1 with ammonia showed a base peak at m/e 553 corresponding to the protonated molecular ion $C_{40}H_{56}OH^+$ (Fig. 2). The proton transfer occurs via the following equilibrium:

$$C_{40}H_{56}O + NH_4^+ \rightleftharpoons C_{40}H_{56}O\text{---}H^+\text{---}NH_3 \rightleftharpoons \underset{m/e\ 553}{C_{40}H_{56}OH^+} + NH_3$$

The spectrum also displayed some very weak peaks with a relative abundance lower than 10%, since only slight fragmentation of the molecule occurs. A molecular weight of 552 was ascribed to DP_1. The electron-impact mass spectrum (Fig. 2) confirms a molecular weight of 552. The other fragmentations may be interpreted as shown in Table 1.

The reaction of 0.1M HCl on DP_1 induced a hypsochromic shift of about 18 nm of the absorption maxima, which demonstrated the presence of one 5,6-epoxide function in the molecule. From all this information, DP_1 was identified as β-carotene-5,6-epoxide.

Identification of DP_2

The chemical ionization mass spectrum with NH_3 showed a peak at m/e 569 which corresponded to the protonated molecular ion $C_{40}H_{56}O_2H$ +. Chemical ionization with CH_4 and electron-impact mass spectra confirmed the molecular weight (568) and the molecular formula ($C_{40}H_{56}O_2$) proposed for DP_2. The electron-impact mass spectrum showed the characteristic fragmentations of epoxides due to the concerted loss of C_6H_8, which occured twice with di-epoxides. Therefore, according to the mass spectral data, DP_2 could include two 5,6-epoxide functions, one 5,6-epoxide and one furanoid-oxide function or two furanoid-oxide functions. The reaction with 0.1M HCl in ethanol induced a 39 nm hypsochromic shift which accounted for the presence of two epoxide functions in the molecule. Thus DP_2 was identified as all *trans*-β-carotene-5,6,5′,6′-diepoxide.

Identification of DP_3

A comparison of the electron-impact mass spectrum of DP_3 with that of DP_1 showed the same molecular weight, the same fragmentation patterns and a comparable relative abundance of the peaks. The only difference was that DP_3 did not react with 0.1M HCl in ethanol, hence it could be positively assimilated to β-carotene-5,8-epoxide, i.e., mutatochrome.

To confirm the three proposed structures, a partial synthesis of the epoxide derivatives was carried out. In the literature, some epoxidation reactions on carotenoids have been described. Carotenoids including β-rings have been epoxidized using monoperphthalic acid (Karrer and Jucker 1945). The formation of β-carotene-5,8-epoxide by treatment of β-carotene with lead tetra-acetate was related by Bordea et al. (1961). It has also been reported that reaction of perbenzoic acid on canthaxanthin provides epoxy derivatives (Goodwin et al., 1956). In the present study, metachloroperbenzoic acid was used. When 0.1 mole of metachloroperbenzoic acid was added to one mole of all trans-β-carotene, TLC separation resulted in three bands with R_f respectively 0.8, 0.7 and 0.5. Band 1 corresponded to unreacted β-carotene. Band 2 was β-carotene-5,6-epoxide and band 3 was β-carotene-5,8-epoxide. These two bands had the same R_f, showed the same UV-Visible spectral data and electron-impact mass spectra as DP_1 and DP_3 respectively.

When 0.2 mole of metachloroperbenzoic acid was added to 1 mole of all trans-β-carotene, TLC separation resulted in three visible bands (B_1, B_3, B_5) and two slightly colored bands (B_2, B_4). B_2 and B_4 corresponded to DP_1 and DP_3 respectively. B_1 was all trans-β-carotene and B_3 which had the same R_f, visible spectral and electronic mass spectral data as DP_2 was β-carotene-5,6,5′,6′-diepoxide. B_5 will be mentioned later in this paper.

Identification of degradation products from all trans-β-carotene during extrusion cooking

Column chromatography of the pigment extracted from the corn starch-pigment mixture gave only fraction A including all trans-β-carotene (95%) and a small amount of *cis* isomers. Thus mixing all *trans*-β-carotene with corn starch and the extracting process did not produce degradation of β-carotene and only induced a few isomerization reactions. TLC separation of fraction B obtained as described in the experimental section, resulted in four bands numbered DP_1', DP_2', DP_3', DP_4' with respectively 0.7, 0.6, 0.5 and 0.4 R_f. The first three compounds were similar to the three degradation compounds identified (DP_1, DP_2, DP_3). According to UV-visible specta, DP_1' and DP_3' probably contain higher relative amounts of *cis* isomers than DP_1 and DP_3: their spectra show hypsochromic shifts of absorption maxima and more intensive *cis* peaks. The infrared spectrum of DP_4' showed that the molecule had a poly-

ene structure and one or several epoxide or furanoid-oxide functions. According to chemical ionization/desorption mass spectrum, the DP_4' molecular weight was 568 and molecular formula was $C_{40}H_{56}O_2$ like DP_3 and DP_3'. Moreover, the electron-impact mass spectra presented similar fragmentation patterns with a comparable relative abundance of the peaks. However, DP_4' reacted with 0.1M HCl to give a hypsochromic shift of 18 nm of absorption maxima which accounted for the presence of only one 5,6-epoxide function in the molecule. Thus, from spectroscopic data, DP_4' was identified as β-carotene-5,6,5',8'-diepoxide.

Its structure was confirmed by chemical epoxidation of all trans-β-carotene which produced band B_5 having the same R_f, visible spectrum and electronic impact mass spectrum as DP_4'.

The isolated compounds were injected separately or as a mixture (fraction B) onto a HPLC column. The chromatograms of fraction B obtained after simple heating or extrusion cooking enabled comparison of the two thermal treatments (Fig. 3 and 4): each peak was identified by its visible spectrum. From these results it appeared that extrusion cooking formed mainly compounds including a furanoid oxide function (β-carotene-5,8-epoxide or β-carotene-5,6,5',8'-diepoxide) and that isomerisation reactions were emphasized. β-carotene-5,6-epoxide was the main component after heating in glass tubes and was present in smaller amounts after extrusion cooking.

In a conjugated system, the terminal double bond has the highest electron density, with this electron density decreasing towards the central double bond. This fact could explain the site of oxygen attack on β-carotene and the epoxidation at the 5,6 positions. Subsequently, a conversion of the epoxide function to a furanoid oxide function can occur. A sequence for the formation of β-carotene epoxides is proposed (Fig. 5), the final product being β-carotene-5,8,5',8'-diepoxide which was also identified after extrusion cooking; its formation will be reported in a later paper. On heating β-carotene at 180°C for 2 hr, only the proximate degradation steps have been demonstrated.

Thus extrusion cooking at 180°C was a more drastic treatment for the pigment than simple heating for a long period (2 hr) at the same temperature. Higher amounts of furanoid oxides and greater degrees of isomerization reactions found in extrusion cooking indicated that degradation of trans-β-carotene was accelerated by vigorous mixing of the paste in the presence of air.

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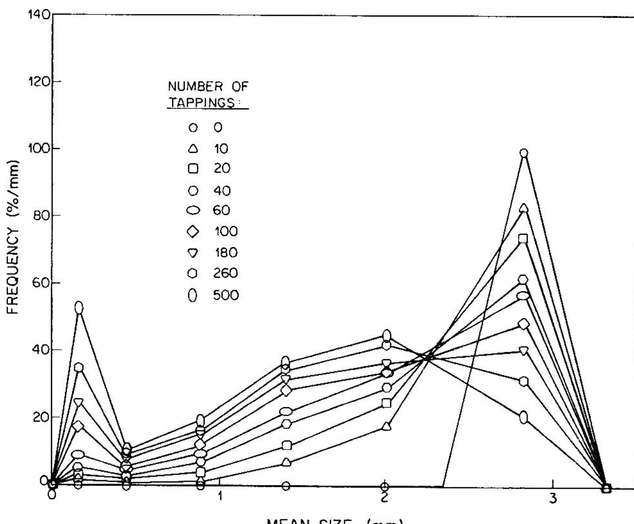

Fig. 11—Particle size distributions of −6+8 mesh fraction of freeze dried coffee subjected to tapping (drop height 40 cm).

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Storage Stability of Soybean Oil-Based Salad Dressings: Effects of Antioxidants and Hydrogenation

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ABSTRACT

Flavor deterioration of salad dressings was investigated to determine the effect of hydrogenation of the oil, additives and storage conditions. Flavor quality tests were developed and correlated with gas chromatographic analyses of volatile compounds in oils separated from the dressings. Hydrogenation of soybean oil with copper and nickel catalysts effectively increased the storage stability of salad dressings at 21°C but not at 32°C. The use of BHA as an antioxidant in the oil or EDTA as a metal inactivator in the starch base as well as nitrogen packaging were effective in prolonging the storage stability of salad dressings made with unhydrogenated soybean oil. Therefore, these additives or nitrogen packaging may provide economic substitutes for hydrogenation of soybean oil used in salad dressings.

INTRODUCTION

SALAD DRESSINGS containing 30–40% vegetable oil account for 35% of the production of all dressings, mayonnaise and sandwich spreads (Swern, 1982). Potential problems in salad dressing manufacture include: separation, discoloration, off-flavor, oxidative rancidity, loss of consistency, and bacterial and fermentation contamination (Young, 1950). The development of rancidity is an important cause of salad dressing deterioration during marketing (Weiss, 1981). The U.S. Army Natick Research & Development Center reported serious problems with salad dressing procurement specifications to avoid deterioration (Bennett, 1982). Common forms of spoilage in salad dressings and mayonnaise may be due to oxidative degradation of the vegetable oil or egg lipids. Therefore, the starting ingredients must be of good initial quality and have good storage stability (Swern, 1982).

A basic ingredient limitation in salad dressing acceptability has been the quality of the vegetable oil. Soybean oil has a 90% share of the prepared dressings market (American Soybean Association, 1978). Extensive research on soybean oil has resulted in marked improvement in oil stability (Cowan, 1965; Frankel, 1980). Important stability factors include: metal inactivation, protection from air, hydrogenation and winterization. Although several researchers have reported that nitrogen packaging of dressings does extend shelflife (Finberg, 1955; Turney, 1963; McCormick, 1967), the effects of oil hydrogenation and metal inactivation in emulsions have not been well documented. Much research has been directed on methods to stabilize vegetable oil systems including hydrogenation and the use of additives. However, little information is available on the effects of these stabilization techniques in food emulsion systems. Porter (1980) has reported significant differences in the effectiveness of antioxidants depending on their use in pure fats or in emulsion systems.

Commercial salad dressings are presently made with both unhydrogenated and hydrogenated soybean oil. Whether or not hydrogenation is necessary to prepare oxidatively stable salad dressings is a controversial issue in the industry. To resolve important questions on the flavor stability of salad dressing emulsions, this study was aimed at (1) determining the effect of hydrogenation of soybean oil to different linolenic acid contents with copper and nickel catalysts, (2) investigating the effects of additives and storage conditions such as temperature, time and inert gas packaging, and (3) correlating sensory data with volatiles analysis by gas chromatography (GC).

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MATERIALS & METHODS

Salad dressing formulation

A Type II starch-based salad dressing was prepared as outlined in Federal Specification EE-M-131G (1979). The ingredient percentages by weight were: 20% distilled water; 21.5% distilled white vinegar (5% acidity); 14% sucrose; 4% starch blend (Dress'n 300, A.E. Staley Co., Decatur, IL); 0.5% sodium chloride; 5% fresh egg yolk and 35% oil. A starch paste, prepared with the first five ingredients, was cooked to 90°C and cooled to 5°C. The yolks were then added and dispersed well. Oil (1.05 kg) was added at approximately 10 ml/min and emulsified with the starch-egg mixture (3 kg) by a Hobart mixer (Model N-50, Troy, OH) with a paddle attachment. Dressings were also prepared under anaerobic conditions by enclosing the mixer in a small plastic chamber under positive nitrogen pressure during the blending of ingredients. The salad dressings met the Federal Specifications' Type II physical requirements for flavor, color, body, texture and emulsion stability (no phase separation after 56 hr at 38°C).

Oil preparation

A single batch of commercially refined and bleached soybean oil was hydrogenated with copper (Cu) catalyst as described by Moulton et al. (1985). The linolenate content of the oil was reduced from 8.7% to 2.4% in the copper hydrogenated soybean oil (CuHSBO-2.4) and to 0.5% in the copper hydrogenated, winterized soybean oil (CuHWSBO-0.5). A commercial sample of nickel (Ni) hydrogenated soybean oil containing 3.3% linolenate was also used. The Cu hydrogenated soybean oil containing 0.5% linolenate (CuHWSBO-0.5) and the Ni hydrogenated soybean oil (NiHWSBO-3.3) were winterized with resulting yields of 83.5% and 75% respectively (Moulton et al., 1985). All oils had cold test values [American Oil Chemists' Society (AOCS) Procedure Cc 11-53, 1981) of 20 hr or above. The three hydrogenated oils and the refined, bleached soybean oil (SBO) were laboratory deodorized (Mounts et al., 1978). Triglycerides were transesterified with $NaOCH_3$ in methanol and the resulting methyl esters were analyzed by gas-liquid chromatography (GLC) on an EGSS-X packed column (Applied Science, State College, PA). Iodine values were calculated on the basis of GLC analyses. The % *trans* was determined by infrared spectrophotometry at 10.3 μm using methyl elaidate as standard according to the AOCS procedure Cd 14-61 (1981). Fatty acid composition and other analyses of the oils are summarized in Table 1.

Additives

All oils were stabilized with 100 ppm citric acid (J.T. Baker Chemical Co., Phillipsburg, NJ), which was added as a 20% aqueous solution on the cooling side of deodorization. Butylated hydroxyanisole (BHA) (Eastman Chemical Products, Inc., Kingsport, TN) was added to a portion of each oil (200 ppm) as a 10% ethanolic solution, also on the cooling side of deodorization. Calcium disodium ethylenediaminetetraacetate (EDTA) (Dow Chemical Co., Midland, MI) was added as a chelatiing agent to a portion of the starch paste (75 ppm).

Table 1—Analyses of soybean oil (SBO), hydrogenated soybean oils (HSBO), and hydrogenated-winterized soybean oils (HWSBO)

Fatty acid analyses	SBO 8.7% Ln[a]	CuHSBO 2.4% Ln	CuHWSBO 0.5% Ln	NiHWSBO 3.3% Ln
C16:0, %	10.1	10.3	9.5	10.1
C18:0	4.3	4.2	4.0	3.8
C18:1	22.7	30.4	41.9	44.6
C18:2	54.2	52.7	44.1	38.2
C18:3	8.7	2.4	0.5	3.3
trans, %	---	8.4	19.4	16.0
Iodine value	136	124	114	113
Cold test, hr	---	> 36	24	20
Winterization yield, %	---	---	83.5	75

[a] Ln = linolenate

Packaging/Storage

The dressings were packaged in 8 oz wide-mouth clear glass bottles with screw-top closures leaving 2 oz of headspace. All samples were stored in duplicate. The dressings made with SBO and with the three hydrogenated soybean oils were prepared and packaged in an air atmosphere. Additional dressings were also prepared with SBO and with NiHWSBO-3.3 in a nitrogen atmosphere. The bottles for these samples were filled and sealed under a constant stream of nitrogen. The dressings were stored for 2 months at 32°C and for 3 and 6 months at 21°C. Controls used in each sensory test were prepared with soybean oil containing citric acid and packaged with air in the headspace and held at 5°C for the length of each storage period.

Sensory evaluations

A 26-member panel, experienced in analytical-descriptive methods (IFT Sensory Evaluation Div., 1981) was trained to differentiate between salad dressing samples of varying flavor qualities and to provide a descriptive analysis of "off" flavor characteristics. Training samples included fresh and aged dressings made with fresh and oxidized soybean oil, hydrogenated soybean oil and cottonseed oil to represent the range of flavor quality and variety of flavor characteristics in dressings. A majority of the panelists were experienced in evaluating flavor quality of vegetable oils. Environmental conditions of the sensory evaluation facility were previously described by Mounts and Warner (1980). The sensory scaling procedure consisted of an unstructured horizontal line with verbal anchors to define the intensity of each flavor characteristic and the overall flavor quality (Eggert, 1982). Numbers on a 1-10 scale were assigned to each point represented on the line with 1 = weak and 10 = strong for the intensity scale for individual flavor characteristics and 1 = bad and 10 = excellent for the overall flavor quality scale. Descriptions of tastes and flavors listed on the scoresheet included: sweet, sour, grassy, rancid, painty, hydrogenated, stale and unidentifiable. These flavor characteristics were selected by the panelists during preliminary sensory tests. Overall flavor quality encompassed all of the perceptible flavor characteristics, which included sweet and sour tastes for each dressing plus any "off" flavors. As the intensity of the "off" flavors increased, the scores for overall flavor quality decreased.

At each testing session, panelists received 5g each of a control dressing, and two aged dressings. All samples were served at 25°C in uncovered paper cups. The control dressing was prepared with soybean oil containing citric acid and held at 5°C (air in headspace) until each evaluation. Preliminary test results showed that the control dressing was consistently rated as good quality. During actual testing, the control was identified as a good quality product but no specific score or point on the scale was indicated. Panelists were instructed to stir the dressing sample as they evaluated the odor of the control first, followed by the experimental samples in the order received. Panelists then tasted the control and rated it for overall quality and individual description intensity. The two experimental samples were evaluated for flavor in the order of the weakest to the strongest in odor. Panelists rinsed their mouths before and between samples with carbon-filtered tap water heated to 38°C.

Instrumental and chemical analyses

To evaluate the dressings for volatiles and peroxide development a procedure was developed to separate the oils from the salad dressings without the use of organic solvents. This separation was achieved by freezing 100g samples at −4°C for 48 hr. The samples were then warmed to 25°C, centrifuged at 9400 × *g* for 15 min and the separated oil was filtered through a 0.5 in. layer of $MgSO_4$ to remove water, and through a layer of $CaCO_3$ to remove acetic acid.

The oil was analyzed for volatile compounds with a Hewlett-Packard 5700 A gas chromatograph (Palo Alto, CA) fitted with a purge-and-trap attachment (Model 7675A). A 1g oil sample was heated to 60°C with the following purge-and-trap settings: 1 min prepurge, 2 min purge, 8 min desorb, 20 mL/min flow and 250°C desorb. The eluted volatiles were trapped on Tenax-GC (Applied Science, Warrenville, IL) and then desorbed and separated on a stainless steel column (6 ft × 1/8 in) packed with Tenax-GC coated with 7% polymetaphenoxylene (Applied Science, Warrenville, IL). The chromatograph was programmed from 70° to 250°C at 8°C/min with injector temperature of 190°C and detector temperature of 250°C. Peaks were identified by matching retention times with those of reference compounds. Peak areas were integrated electronically. Peroxide values (me/kg) were determined on the separated oil according to the standard AOCS procedure Cd 8-53 (1981).

Microbiological analyses

Diluted aged salad dressings were plated on three types of agar specific for yeasts/molds, aerobic bacteria and lactobacillus, respectively: (1) yeast-malt agar containing tetracycline-HCl; (2) plate count agar (PCA) (Becton, Dickinson and Co., Cockeysville, MD) containing cycloheximide; and (3) Rogosa SL agar (Difco, Detroit, MI) (Kurtzman et al., 1971; Kurtzman and Smittle, 1984). The plated dressings were also examined microscopically for dead cells to determine whether or not viable organisms were present at one time in the samples.

Statistical analyses

A multifactorial design was developed to evaluate the effects of the following four parameters: linolenate at four levels in the oil; three antioxidants/metal chelators; two storage atmospheres and four time-temperature storage periods, for a total of 72 observations. In the first set of dressings, prepared and packaged in air, the effects of four levels of linolenate were compared within each additive treatment group at each storage period. In the second set of dressings, prepared and packaged under nitrogen, the effects of air vs nitrogen atmosphere were compared for two of the linolenate level groups (SBO and NiHWSBO) within each additive group at each storage period. Analysis of variance, linear regression and correlation coefficients were calculated for the sensory data and volatiles analyses (Cochran and Cox, 1957). For sensory data, statistically significant differences in scores were determined by calculating least significant differences (LSD). For GC analyses of volatiles, which showed peak areas ranging widely by integrator counts, a least significant ratio (LSR) was calculated (Snedecor, 1956). All differences indicated as statistically significant were at the 95% confidence level ($P<0.05$).

RESULTS

TO ENSURE that the oils used in these storage stability tests of salad dressings were of good flavor quality, the oil samples were evaluated for flavor and oxidative stability prior to formulation of dressings. All oils received initial scores, indicative of good flavor quality, ranging from 8.4 for the CuHWSBO-0.5 to 7.1 for the NiHWSBO-3.3. Peroxide values for the initial oils were zero. After storage for 8 days at 60°C, the oils ranged in score from 6.6 for the NiHWSBO to 5.1 for the SBO containing BHA compared to 5.3 for the SBO with citric acid only.

The dressings in this study had pH values ranging from 3.3–3.5, which is comparable to commercial dressings that have a pH ranging from 3.2–3.9 (Smittle, 1977). No microbial colonies were detected on the plated samples. Microscopic evaluation of the dressings showed no dead cells present. Therefore, any "off" flavors detected by the panelists were not indicative of microbial spoilage. This observation is in agreement with those of Kurtzman et al. (1971) and Smittle (1977) who reported that microbial growth usually occurs in dressings with a pH range of 3.6–4.1.

Sensory evaluation

To evaluate the aged salad dressings for flavor quality, the panelists were required to note development of "off" flavor characteristics in addition to changes in the intensities of sweet and sour taste. The predominant "off" flavors were stale and

Table 2—Effects of hydrogenation, additives, and storage in air on overall flavor quality scores[a] of salad dressings

Oil[b]	Additive[c]	Initial	Storage time/temperature 2 mo 32°C	3 mo 21°C	6 mo 21°C
SBO					
	None	8.0	5.7	5.6	6.6
	BHA	8.0	5.5	7.3	6.5
	EDTA	7.8	6.6	6.7	6.9
CuHWSBO-0.5					
	None	7.7	5.1	6.8	6.5
	BHA	7.7	6.0	6.6	6.0
	EDTA	8.1	6.6	6.4	6.6
CuHSBO-2.4					
	None	7.8	5.6	6.5	7.2
	BHA	8.0	5.5	7.2	6.6
	EDTA	7.9	6.1	6.4	7.1
NiHWSBO-3.3					
	None	7.8	5.6	6.4	6.7
	BHA	7.7	6.4	7.1	7.0
	EDTA	8.0	6.7	7.3	7.0

[a] Least significant difference (LSD) = 0.7

[b] Oil identification = SBO (Soybean Oil); CuHWSBO-0.5 (Copper hydrogenated, winterized SBO-0.5% linolenate); CuHSBO-2.4 (Copper hydrogenated SBO-2.4% linolenate); NiHWSBO-3.3 (Nickel hydrogenated, winterized SBO-3.3% linolenate)

[c] All oils contain 100 ppm citric acid

rancid, but descriptions such as grassy, hydrogenated and painty were also used occasionally.

Effect of hydrogenation. Flavor quality scores were compared on the dressings prepared with the four oils containing only citric acid and processed in air. Initial quality of the dressings showed no significant effect of hydrogenation (Table 2). Scores ranged from 7.7–8.1 for all samples compared to 8.0 for the control dressing (LSD = 0.7). The dressings were described as slightly more sour than sweet with an average sour taste intensity of 7.1 and an average sweet taste intensity of 6.1. Few "off" flavors were reported.

After storing dressings in air for 2 months at 32°C and for 3 and 6 months at 21°C the flavor quality scores were all significantly decreased relative to the control dressing (Table 2). Scores ranged from 5.1–6.7 for dressings after 2 months at 32°C; from 5.6–7.3 after 3 months at 21°C and from 6.0–7.2 after 6 months at 21°C, indicating poor to good quality. Taste intensities for sweet and sour generally decreased in the salad dressings with increasing storage time (Table 3). The decreases may be attributed to a mellowing or blending of these two tastes as well as to the development of "off" flavors which could mask the sweetness and sourness. After the 3-month storage, dressings made with hydrogenated oils had significantly higher quality scores than the sample prepared with unhydrogenated oil because of the significantly higher intensity of rancid flavor in the dressing made with unhydrogenated oil. No significant differences in scores were noted among the three hydrogenated oils. After 6 months of storage at 21°C and 2 months at 32°C, dressings prepared with hydrogenated oils were rated as not significantly different than the dressings made with unhydrogenated oil. Dressings aged for 2 months had the highest stale and rancid flavor intensities.

Effect of additives. Flavor quality scores were compared on dressings prepared with the same oil type but with different additives. Quality scores of the dressings aged for 2 or 3 months were significantly improved in most samples containing BHA or EDTA compared with dressings containing only citric acid (Table 2). The most pronounced effect of EDTA was noted after 2 months storage at 32°C. The use of EDTA prevented significant deterioration in dressings made with SBO, CuHWSBO-0.5 and NiHWSBO-3.3. After 3 months of storage at 21°C, dressings made with SBO and EDTA and with NiHWSBO-3.3 and EDTA were rated significantly higher in quality than samples made with these oils containing only citric acid. After 2 months of storage at 32°C, the dressing prepared with SBO and EDTA had a significantly higher flavor quality score than all dressings made with hydrogenated oils and citric acid only. Therefore, EDTA was more effective than hydrogenation in preventing flavor deterioration under this severe storage condition. However, after 3 months of storage at 21°C, EDTA was as effective as hydrogenation in protecting dressing quality. Compared with citric acid only, the use of BHA improved the flavor quality of several dressings including those made with: NiHWSBO-3.3 and CuHWSBO-0.5 after storage for 2 months at 32°C and SBO, NiHWSBO-3.3 and CuHSBO-2.4 after 3 months at 21°C. After 6 months storage at 21°C, dressings prepared with either EDTA or BHA showed no significant differences from dressings prepared with oils treated with citric acid only. No synergistic effect of hydrogenation and additives occurred at any of the storage conditions in air. The use of EDTA decreased the intensities of stale and rancid flavors in salad dressings aged in air at the three storage times (Table 3). However, statistically significant differences were observed in only a few comparisons. Dressing prepared with SBO and with EDTA and aged 2 months at 32°C not only had significantly lower intensities of stale and rancid flavors than the dressing made with CuHSBO-2.4 containing only citric acid, but also a lower intensity of stale flavor than the sample prepared with CuHWSBO-0.5. The use of BHA caused significant decreases in "off" flavor intensities in dressings prepared with either NiHWSBO-3.3 or SBO, containing only citric acid and aged in air for 3 months at 21°C. Although the use of EDTA was more effective than hydrogenation in preventing deterioration in dressings aged 2 months, both methods enhanced the stability of the dressings aged at 3 months.

Effect of nitrogen atmosphere. Flavor quality scores were compared on dressings prepared and packaged in an air atmosphere vs. dressings made with the same oil type and additives under a nitrogen atmosphere. Dressings made with SBO received higher flavor quality scores after 2 and 3 months of storage under nitrogen than dressings processed and stored in air (Table 4). This result was probably due to significant differences in rancid flavor intensities (Table 3). Under nitrogen atmosphere, dressings made with either SBO or NiHWSBO-3.3 oil plus BHA had significantly higher flavor scores than similar samples processed and aged in air. In several stabilized samples, a nitrogen atmosphere resulted in lower scores than for corresponding dressings processed in air. Dressings containing EDTA and aged for 2 months at 32°C under nitrogen were rated lower than equivalent samples aged in air. All samples aged for 3 months at 21°C under nitrogen were rated lower than the same dressings processed in air except for the dressing made with SBO and citric acid only. No significant differences between air and nitrogen packaging were noted for the dressings aged 6 months at 21°C. Therefore, preparation and storage of salad dressings under nitrogen were of benefit when NiHWSBO and SBO were used alone or with BHA after 2 months storage. Nitrogen atmosphere was also of benefit in dressings made with SBO containing only citric acid and aged 3 months.

Analysis of volatiles by gas chromatography

The gas chromatogram of volatile compounds, obtained with a purge-and-trap technique using a packed column, showed 12 peaks consistently appearing in oils separated from the salad dressings. Major volatile compounds included pentane and hexanal (Tables 5-7). Minor volatiles were pentanal, heptanal and 2,4-decadienal. In previous work (Fore et al., 1978; Legendre et al., 1980; Min and Tickner, 1982) done by the direct injection technique, the same volatiles were also reported in salad dressings but larger amounts of 2,4-decadienal were observed. Previous work by Snyder and Frankel (1985) on volatile analyses of oils by a static headspace technique showed that low-boiling compounds such as pentane and hexanal were detected in much larger amounts than high-boiling compounds such as 2,4-decadienal. Least significant ratios (LSR) were calculated to facilitate comparisons ($P<0.05$) between integrator counts of total volatiles listed in Table 5. High LSR values

Table 3—Flavor characteristics of aged salad dressings

			Flavor characteristics[b,c]											
			Sweet			Sour			Stale			Rancid		
Oil[a]	Additive	Headspace	2 mo 32°C	3 mo 21°C	6 mo 21°C	2 mo 32°C	3 mo 21°C	6 mo 21°C	2 mo 32°C	3 mo 21°C	6 mo 21°C	2 mo 32°C	3 mo 21°C	6 mo 21°C
SBO	None	Air	5.6	5.5	5.1	6.3	6.5	6.0	2.2	1.0	1.0	1.9	1.5	0.3
	BHA	Air	5.6	5.4	5.9	6.5	6.7	6.5	1.7	0.4	0.3	0.6	0	0.5
	EDTA	Air	5.7	4.9	5.9	6.4	6.5	6.5	0.7	0.9	0.5	0.4	0	0
SBO	None	N_2	5.2	5.5	5.7	6.7	6.9	6.4	1.5	1.0	0.8	0	0	0
	BHA	N_2	5.7	5.3	5.5	6.9	6.6	6.8	1.2	1.1	0.8	0.7	0.7	0
	EDTA	N_2	5.5	5.5	5.1	7.1	6.7	6.2	1.3	0.6	0.6	0.4	0	0.5
CuHWSBO-0.5	None	Air	5.5	5.6	5.7	6.3	7.2	6.5	1.7	1.3	1.2	0.7	0	0.2
	BHA	Air	5.2	5.5	6.0	5.9	6.7	6.8	1.1	0.6	1.2	0.6	0	0.4
	EDTA	Air	6.0	5.9	5.6	7.0	6.8	6.6	1.4	1.0	0.8	0.2	0	0
CuHSBO-2.4	None	Air	5.7	5.6	5.6	6.9	6.4	6.4	2.2	1.1	0.7	1.4	0.4	0
	BHA	Air	4.6	5.4	5.8	6.3	6.8	6.2	1.5	0.8	0.7	1.1	0	0
	EDTA	Air	6.3	5.1	6.5	6.4	6.5	7.0	1.7	0.4	0.5	0.3	0	0
NiHWSBO-3.3	None	Air	5.5	5.6	5.5	6.3	6.8	6.5	1.1	1.6	0.8	0.6	0.5	0
	BHA	Air	6.1	5.5	6.1	6.9	7.0	6.9	1.1	0.6	0.7	0.9	0	0
	EDTA	Air	5.4	5.9	5.7	7.0	6.7	6.2	1.0	0.6	0.3	0	0	0
NiHWSBO-3.3	None	N_2	6.2	5.5	5.6	7.2	7.0	6.6	1.5	0.8	1.1	0	0.3	0.7
	BHA	N_2	5.9	5.1	5.7	7.1	6.7	7.1	1.4	0.7	0.6	0.2	0.2	0
	EDTA	N_2	5.6	5.9	4.8	6.9	6.6	6.3	1.8	0.6	0.9	0.4	0	0.9

[a] Oil identification = SBO (Soybean Oil); CuHWSBO-0.5 (Copper hydrogenated, winterized SBO-0.5% linolenate); CuHSBO-2.4 (Copper hydrogenated SBO-2.4% linolenate); NiHWSBO-3.3 (Nickel hydrogenated, winterized SBO-3.3% linolenate)
[b] SBO Control = 6.1, sweet; 7.1, sour; no "off" flavors
[c] Least significant difference (LSD) = 1.0

Table 4—Effects of storage in air or nitrogen on flavor quality scores[a] of salad dressings

			Storage time/temperature			
Oil[b]	Additive[c]	Headspace	Initial	2 mo 32°C	3 mo 21°C	6 mo 21°C
SBO						
	None	Air	8.0	5.5	5.6	6.6
		N_2		6.2	6.8	6.7
	BHA	Air	8.0	5.3	7.3	6.5
	BHA	N_2		6.0	6.4	7.0
	EDTA	Air	7.8	6.6	6.7	6.9
	EDTA	N_2		6.2	6.3	6.5
NiHWSBO-3.3						
	None	Air	7.8	5.6	6.4	6.7
		N_2		6.6	5.9	6.5
	BHA	Air	7.7	6.0	7.1	7.0
	BHA	N_2		6.7	6.3	6.9
	EDTA	Air	8.0	6.7	7.3	7.0
	EDTA	N_2		6.1	7.1	6.1

[a] Least significant difference (LSD) = 0.7
[b] Oil identification = SBO (Soybean Oil); NiHWSBO-3.3 (Nickel hydrogenated, winterized SBO-3.3% linolenate)
[c] All oils contain 100 ppm citric acid

Table 5—Effect of storage in air and nitrogen on total GC volatiles[a] in oils isolated from salad dressings

			Storage time/temperature			
Oil[b]	Additive[c]	Headspace	Initial	2 mo 32°C	3 mo 21°C	6 mo 21°C
SBO						
	None	Air	10	30	42	138
		N_2	(10)[d]	5	35	64
	BHA	Air	26	21	37	67
		N_2	(26)[d]	10	41	119
	EDTA	Air	2	16	36	69
		N_2	(2)[d]	9	31	78
CuHWSBO-0.5						
	None	Air	3	22	27	94
	BHA	Air	36	35	26	85
	EDTA	Air	7	26	21	75
CuHSBO-2.4						
	None	Air	5	47	20	81
	BHA	Air	20	31	25	71
	EDTA	Air	21	18	36	23
NiHWSBO-3.3						
	None	Air	28	50	28	97
		N_2	(28)[d]	15	38	126
	BHA	Air	25	33	15	74
		N_2	(25)[d]	2	61	121
	EDTA	Air	12	11	19	34
		N_2	(12)[d]	2	34	163

[a] Integration units × 10^4, mean values for duplicate determinations. Least significant ratio (LSR) = 1.2 (P<0.05). Relative standard deviation = ±7.7%.
[b] Oil identification = SBO (Soybean Oil); CuHWSBO-0.5 (Copper hydrogenated, winterized SBO-0.5% linolenate); CuHSBO-2.4 (Copper hydrogenated SBO-2.4% linolenate); NiHWSBO-3.3 (Nickel hydrogenated, winterized SBO-3.3% linolenate)
[c] All oils contain 100 ppm citric acid
[d] Assumed same as in air

(≥1.2) indicated a significant effect of experimental treatments in decreasing total volatiles relative to control samples (Table 6).

After storage at 21°C for 3 and 6 months, dressing prepared with hydrogenated oils formed significantly less total volatile compounds than dressings made with unhydrogenated oil (Table 5). The LSR values ranged from 1.4 to 2.1 for comparisons between aged dressings made with SBO (Control) and with NiHWSBO-3.3 aged 6 months (LSR = 1.4) or CuHSBO-2.4 aged 3 months (LSR = 2.1) (Table 6). However, this stabilizing effect of hydrogenation was only observed after 2 months storage in the dressing made with CuHWSBO-0.5 (LSR = 1.4). These results follow the same trend as the flavor quality evaluations of dressings aged 2 months at 32°C and 3 months at 21°C (Table 2). Linear regression analysis of flavor quality scores and total volatiles in the samples containing citric acid only showed correlation coefficients of −0.20, −0.70, and −0.54 for dressings aged in air 2 months at 32°C, 3 months at 21°C and 6 months at 21°C, respectively. Therefore, as in flavor evaluation results, storage for 3 months was the most sensitive condition to show the effect of hydrogenation on the stability of these dressings based on total volatile analyses.

The use of EDTA significantly decreased the amount of volatiles in most dressings compared with control samples containing citric acid only (Tables 5 and 6). Two exceptions in

Table 6—Comparisons of total GC volatiles based on calculated ratios between control and experimental salad dressings (data from Table 5)

Sample treatments		Ratios[a] of integrator counts of oils[b] in dressings (Control:Experimental)											
		SBO			CuHWSBO-0.5			CuHSBO-2.4			NiHWSBO-3.3		
Control	Experimental	2 mo 32°C	3 mo 21°C	6 mo 21°C	2 mo 32°C	3 mo 21°C	6 mo 21°C	2 mo 32°C	3 mo 21°C	6 mo 21°C	2 mo 32°C	3 mo 21°C	6 mo 21°C
SBO[c]	Hydrogenated Oil[c]				1.4	1.5	1.5	0.6	2.1	1.7	0.6	1.5	1.4
Citric Acid	BHA	1.4	1.2	2.1	0.6	1.0	1.1	1.5	0.8	1.1	1.5	1.5	1.3
Citric Acid	EDTA	1.9	1.2	2.0	0.9	1.3	1.3	2.6	0.6	3.5	4.5	1.5	2.9
Air	N_2												
+ Citric Acid	+ Citric Acid	6.0	1.2	2.2	---[d]	---	---	---	---	---	3.3	0.7	0.8
+ BHA	+ BHA	2.1	0.9	0.6	---	---	---	---	---	---	16.5	0.2	0.6
+ EDTA	+ EDTA	1.8	1.2	0.9	---	---	---	---	---	---	5.5	0.6	0.2

[a] Least Significant Ratio (LSR) ≥1.2 ($P<0.05$) between control and experimental treatments.
[b] Oil Identification = SBO (Soybean Oil); CuHWSBO-0.5 (Copper hydrogenated, winterized SBO-0.5% linolenate); CuHSBO-2.4 (Copper hydrogenated SBO-2.4% linolenate); NiHWSBO-3.3 (Nickel hydrogenated, winterized SBO-3.3% linolenate)
[c] + Citric Acid; in air
[d] --- not tested

Table 7—Effect of storage in air and nitrogen on pentane[a] plus hexanal[a] and peroxide values in oils isolated from salad dressings

			Pentane + hexanal				Peroxide value (me/kg)			
Oil[b]	Additive[c]	Headspace	Initial	2 mo 32°C	3 mo 21°C	6 mo 21°C	Initial	2 mo 32°C	3 mo 21°C	6 mo 21°C
SBO										
	None	Air	4	14	24	63	1	18	15	20
	BHA	Air	21	12	20	32	1	8	6	31
	EDTA	Air	2	10	20	56	---[d]	9	4	12
SBO										
	None	N_2	(4)[e]	2	21	32	---	16	12	25
	BHA	N_2	(21)[e]	7	26	69	---	18	22	29
	EDTA	N_2	(2)[e]	6	21	62	---[d]	6	2	11
CuHWSBO-0.5										
	None	Air	1	15	13	47	0	7	17	27
	BHA	Air	23	24	17	44	0	11	18	33
	EDTA	Air	4	21	15	54	0	8	2	12
CuHSBO-2.4										
	None	Air	1	15	12	44	0	15	19	32
	BHA	Air	14	21	16	38	0	11	16	30
	EDTA	Air	13	15	28	24	0	11	8	15
NiHWSBO-3.3										
	None	Air	18	12	29	75	0	11	14	21
	BHA	Air	20	1	33	55	0	5	14	20
	EDTA	Air	6	2	15	99	0	6	4	7
NiHWSBO-3.3										
	None	N_2	(18)[e]	12	29	75	---	5	11	21
	BHA	N_2	(20)[e]	1	33	75	---	10	13	21
	EDTA	N_2	(6)[e]	2	24	99	---	1	1	4

[a] Integration units × 10^4, mean values for duplicate determinations. Least significant ratio (LSR) = 1.2 ($P<0.05$). Relative standard deviation = ±7.7%
[b] Oil identification = SBO (Soybean Oil); CuHWSBO-0.5 (Copper hydrogenated, winterized SBO-0.5% linolenate); CuHSBO-2.4 (Copper hydrogenated SBO-2.4% linolenate); NiHWSBO-3.3 (Nickel hydrogenated, winterized SBO-3.3% linolenate)
[c] All oils contain citric acid
[d] --- not tested
[e] Assumed same as in air

the effect of EDTA were for a dressing made with CuHWSBO-0.5 and aged 2 months at 32°C (LSR = 0.9) and for a sample prepared with CuHSBO-2.4 and stored 3 months at 21°C (LSR = 0.6). Correlation coefficients between flavor scores and total volatile compounds in dressings with EDTA were: −0.36, after the 2-months storage at 32°C; −0.78, after 3 months storage at 21°C, and −0.75 after the 6-months storage at 21°C. Therefore, volatiles analysis of the oils isolated from the dressings aged 3 and 6 months at 21°C was the most sensitive monitor of the effect of EDTA on quality. The use of BHA significantly decreased volatile formation in dressings prepared with either SBO or NiHWSBO-3.3 (LSR range 1.2-1.5) (Tables 5 and 6) compared to dressings containing citric acid only. However, BHA was no more effective than citric acid in dressings prepared with CuHWSBO-0.5 or CuHSBO-2.4. Only at the more severe storage of 2 months, 32°C did BHA prevent more volatiles formation in CuHSBO-2.4 (LSR = 1.5) than did citric acid.

Nitrogen had a significant effect in decreasing volatile formation in dressings prepared with SBO compared to similar samples prepared in air. In the samples containing citric acid only, the LSR values ranged from 1.2 to 6.0 for the three storage periods (Table 6). The use of nitrogen prevented significant volatile formation in dressings made with SBO and BHA only after storage of 2 months (LSR = 2.1); whereas the effect was significant for dressings made with SBO and EDTA at both the 2 month and 3 month storage periods. Nitrogen also significantly decreased volatiles in dressings prepared with NiHWSBO-3.3 and aged at 2 months (LSR values 3.3–16.5). However, more volatiles were formed in dressings prepared with NiHWSBO-3.3 in a nitrogen atmosphere than in air after storage at 3 and 6 months. These results are consistent with the negative effect of nitrogen atmosphere observed with flavor quality scores (Table 4).

Aged dressings containing BHA or EDTA and packaged under nitrogen had some extraneous, unidentified peaks in the gas chromatograms. To determine whether or not these peaks affected analyses of total volatiles, peak areas for pentane and hexanal were added separately (Table 7). These results showed similar trends as those based on total volatiles (Table 5). With salad dressings made with SBO, storage under nitrogen decreased the combined total peak area of pentane and hexanal. However, in the presence of additives, storage under nitrogen showed either no effect or an actual increase in pentane and

hexanal. Correlation coefficients between flavor scores and hexanal contents were comparable to the coefficients calculated between scores and total volatiles. On the other hand, correlation coefficients between scores and pentane were not significant.

Peroxide values

Peroxide value determinations in oils isolated from dressings varied initially from 0–1 for the hydrogenated and unhydrogenated oils (Table 7). Hydrogenation was only effective in decreasing peroxide values after 2 months of storage at 32°C. Little difference was observed between the dressings with unhydrogenated oil and those with hydrogenated oils after 3 and 6 months of storage. These results were observed for samples packaged in either air or nitrogen. The effect of hydrogenation in lowering peroxide values after the 2-month storage is in contrast to the flavor evaluations (Table 2) and total volatile analyses (Table 5), which show no effect from hydrogenation. Therefore, we found no relation between peroxide values of dressings made with hydrogenated and nonhydrogenated oils and results based upon flavor and volatile analyses.

The use of EDTA was effective in lowering peroxide values in all four oil types and at all storage times and temperatures with dressings prepared and packaged in air (Table 7). On the other hand, BHA had a limited effect with only dressings made with SBO and aged either 2 months at 32°C or 3 months at 21°C and in dressing made with NiHWSBO-3.3 and aged 2 months at 32°C. We found EDTA prevented increases in peroxide values more effectively than either hydrogenation or BHA.

In the presence of BHA, dressings packaged under nitrogen had higher peroxide values than similar samples packaged in air. In the presence of citric acid only or EDTA, peroxide values were generally lower for those dressings packaged under nitrogen than in air. The use of nitrogen packaging was equally effective in SBO and in the NiHWSBO-3.3 when dressings contained either EDTA or citric acid.

DISCUSSION

SALAD DRESSINGS are multiphase emulsion systems that undergo complex interactions on storage in air. Therefore, simple, straightforward correlations may not be expected between sensory analyses and chemical or instrumental tests such as peroxide values and gas chromatographic volatile analyses. Mounts et al., (1978, 1981) reported that hydrogenation did not improve the flavor stability of soybean oil either in the presence or absence of BHA. However, oxidative stability measured by peroxide values was improved with either hydrogenation or BHA. The lack of effectiveness in the flavor stability of oils by hydrogenation and BHA (Mounts et al., 1978) is in contrast to this present study with salad dressing emulsions showing a positive effect from hydrogenation and/or BHA. However, our results with dressings are consistent with previous reports that BHA is more active in stabilizing emulsion systems than bulk vegetable oil systems (Porter, 1980). According to this theory, since BHA is very lipophilic, it would be more effective in emulsions because more of the antioxidant is located close to the boundary layer in the lipid miscelles, where oxidation is most intense.

This study showed that hydrogenation of soybean oil effectively increased the storage stability of salad dressings at 21°C but not at 32°C. The use of BHA as an antioxidant in the oil or EDTA as a metal inactivator in the starch base as well as nitrogen packaging were effective in prolonging the storage stability of salad dressings made with unhydrogenated soybean oil. No synergistic effect was noted from hydrogenation combined with additives or nitrogen packaging. Therefore, the use of antioxidants, EDTA, or nitrogen packaging may provide economic substitutes for hydrogenation of soybean oil used in salad dressings.

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Evaluation of Thermal Processing of Retortable Pouches Filled with Conduction Heated Foods Considering their Actual Shapes

SHWETA TANDON and SANTI R. BHOWMIK

ABSTRACT

A computer model was developed to evaluate thermal processing of a retortable pouch containing a conduction heated food. In this model, a transient 2-dimensional heat conduction equation was solved using a modified finite difference technique for a food-filled retortable pouch considering its actual shape. The model was used to determine process time, mass average sterilizing value and nutrient retention to achieve a prefixed level of lethality in the pouched product for a given temperature profile of the heating medium. Temperature distributions and sterilizing values predicted by the developed model were compared with similar values obtained by applying a finite element technique with the aid of a package program (ANSYS). Close agreements were found between the developed model and the finite element technique.

INTRODUCTION

RESEARCH INTEREST in the evaluation of thermal processing of retortable pouches was stimulated by its approval for use in commercial sterilization of low acid foods. Numerous articles were published describing the benefits of using the retortable pouches in food processing (Mermelstein, 1978; Lampi, 1980). Several studies were undertaken to develop methods to evaluate thermal processing of retortable pouches containing conduction heated foods.

Ohlsson (1980) presented a numerical solution of the Fourier's heat conduction equation in one dimension to obtain an optimal temperature profile for the retortable pouches and to achieve a minimum loss in sensory and nutritional quality in the processed food. The geometrical configuration of the pouch was assumed to be an infinite slab to conveniently apply finite difference technique.

Castillo et al. (1980) developed an analytical method to predict the critical point sterilizing value and nutrient retention in conduction heated foods thermally processed in a retortable pouch. They assumed the pouch to be a rectangular parallelopiped. Nutrient degradation during come-up and cooling time of the process cycle was neglected. The model showed 2–16% deviation between predicted and experimental nutrient retention values. Manson et al. (1970) developed a method to evaluate thermal processing of conduction heated foods in rigid rectangular containers by solving the differential heat conduction equation in three dimensions using a finite difference technique. The time-temperature history estimated for different locations (nodal points) inside the container was used to predict lethality and nutrient retention during thermal processing.

Hayakawa (1977) developed computerized models for estimating proper thermal processes of canned foods based on a formula method. This model can be applied to retortable pouches subject to thermal processing at a constant retort temperature.

All these methods available in the literature are applicable to rectangular containers. However, the retortable pouch is flexible and the filled pouch when placed in a cassette during thermal processing conforms to an anomalous shape similar to a pillow. The assumption of a regular rectangular or parallelopiped configuration is necessary to apply the numerical (finite difference) techniques for thermal process evaluation. These assumptions led to overestimation of process time resulting in less retention of nutrients and greater loss of sensory quality in the food.

The finite element technique is applicable to objects having any shape or size and is used in studying freezing and defrosting of foods (Rebellato et al., 1978). Some researchers (Singh and Segerlind, 1974) have successfully used this technique to estimate transient temperatures of canned foods and chicken legs undergoing heat treatment. Naveh et al. (1983) applied the finite element method to evaluate thermal processing of conduction heated foods in glass jars. The objective of this investigation was to develop a modified finite difference technique for evaluating thermal processing of the institutional size, retortable pouch by considering its actual shape and comparing this method with another model based on the finite element technique.

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THEORY

Development of modified finite difference model (FDM)

The transient, isotropic heat conduction in an institutional size retortable pouch containing conduction heated foods can be represented by the following Fourier's equation in two dimensions:

$$\frac{1}{\alpha}\frac{\partial T}{\partial t} = \frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} \quad (1)$$

where, T = temperature as a function of x, y and t; t = time; α = thermal diffusivity of food.

Considering heat transfer through the cross-section of the pouch (Fig. 1), the finite difference technique, which approximates the derivatives of a function at a point in terms of its value at several neighboring points, was applied to solve the above equation as the following:

$$\frac{T_{i,j,n} - T_{i,j,n-1}}{\Delta t} = \alpha\,[T_{i+1,j,n} - 2T_{i,j,n} + T_{i-1,j,n}] / \Delta x^2 + \alpha\,[T_{i,j+1,n} - 2T_{i,j,n} + T_{i,j-1,n}] / \Delta y^2 \quad (2)$$

where, i and j signify the location of nodes; n signifies time level; Δx is increment of length in x-direction; Δy is increment of length in y-direction.

The cross-section of a food filled retortable pouch was divided into a finite set of discrete elements by superimposing an x-y grid pattern (Fig. 1). To resolve problems of instability and minimize the number of iterations, the Alternating Direction Implicit (ADI) method (Ames, 1977; Douglas, 1955) was used to solve Eq. (2). The ADI method involves the alternate use of two different finite difference equations, one implicit only in the x-direction and the other implicit only in the y-direction, applied over successive time intervals of duration, $\Delta t/2$.

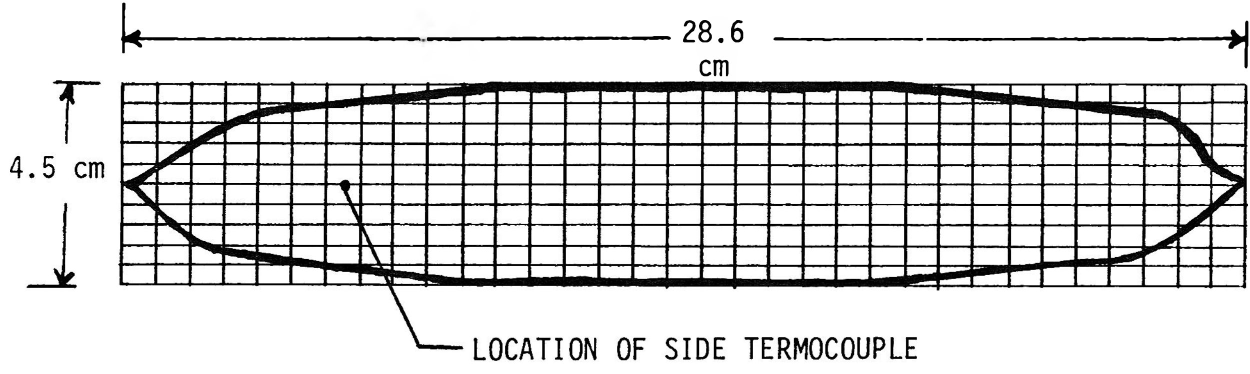

Fig. 1 — Largest cross-section of a food filled pouch.

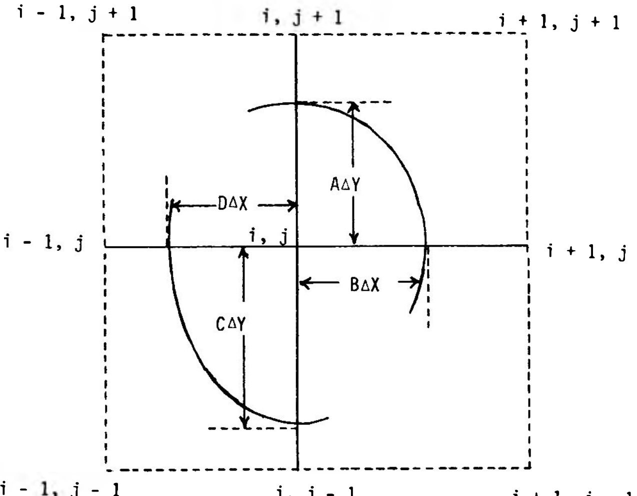

Fig. 2 — Pictorial representation of proportionality constants.

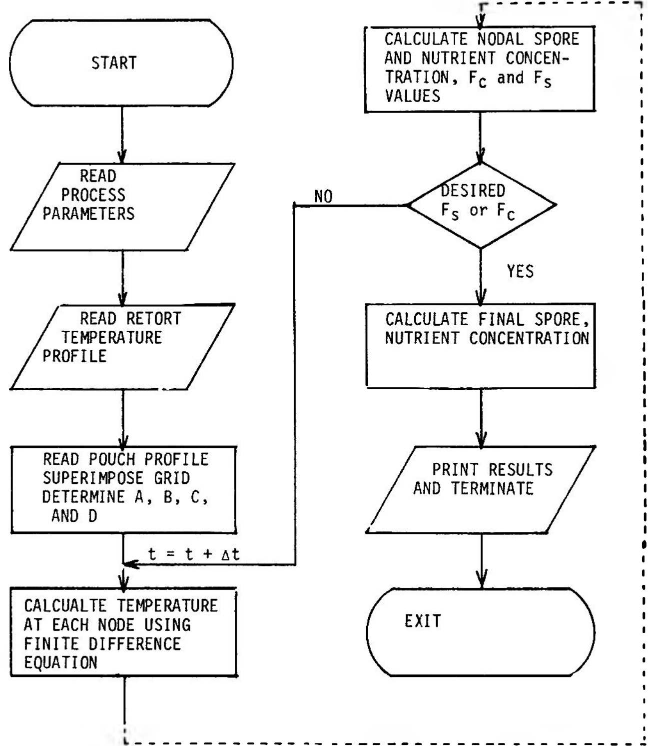

Fig. 3 — Abridged flow diagram for thermal process evaluation by the modified finite difference technique.

Table 1 — Process parameters used in computer simulation

C_o	=	100,000
C_{no}	=	100%
T_i	=	71.1°C
α	=	0.1903×10^{-6} m²/sec
z	=	10.0 C°
z_n	=	31.0 C°
D_r	=	1.0 min
D_{rn}	=	140.4 min
Δt	=	0.125 min
T_r	=	119°C

Due to the irregular shape of the retortable pouch, the boundary intersects the grid pattern at locations other than nodes (Fig. 1). In this situation, the distance between the boundary and its adjacent internal node point is some factor of Δx or Δy. These fractional distances are described by proportionality constants A, B, C, and D as shown in Fig. 2. To consider the effect of irregular boundary on the temperature response of the food inside the pouch, the finite difference equations were modified using Taylor's series (Carnahan et al., 1969) and incorporating the above proportionality constants. The modified equation for an irregular shape pouch applicable to internal node points implicit in the x-direction is given by:

$$T^*_{i,j} - T_{i,j,n} = \frac{\alpha\Delta t[BT^*_{i-1,j} - (D+B)T^*_{i,j} + DT^*_{i+1,j}]}{BD(B+D)\Delta x^2} + \frac{\alpha\Delta t[AT_{i,j-1,n} - (A+C)T_{i,j,n} + CT_{i,j+1,n}]}{AC(A+C)\Delta y^2} \quad (3)$$

while the corresponding equation in the y-direction is:

$$T_{i,j,n+1} - T^*_{i,j} = \frac{\alpha\Delta t[BT^*_{i-1,j} - (D+B)T^*_{i,j} + DT^*_{i+1,j}]}{BD(B+D)\Delta x^2} + \frac{\alpha\Delta t[AT_{i,j-1,n+1} - (A+C)T_{i,j,n+1} + CT_{i,j+1,n+1}]}{AC(A+C)\Delta y^2} \quad (4)$$

For all situations, the proportionality factors have values between zero and one.

At Surface Node Points:

$$T_{i,j,n} = T_m \quad (5)$$

where T^* is the intermediate value of temperature at the end of the first half-time step and T_r is the retort temperature. The derivation of the above equations is shown in the Appendix.

A transient boundary condition was achieved in the computer model by varying the retort temperature profile at each

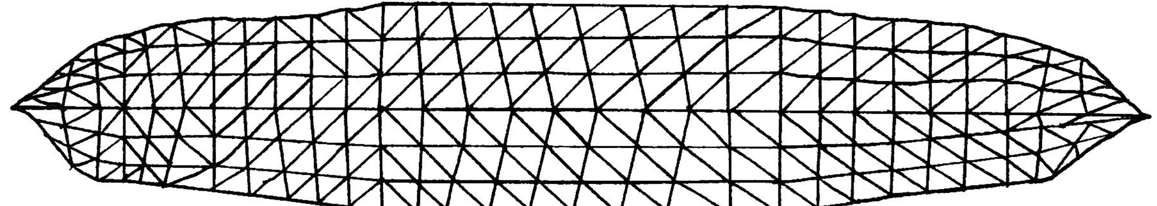

Fig. 4 — Retort pouch profile for finite element model.

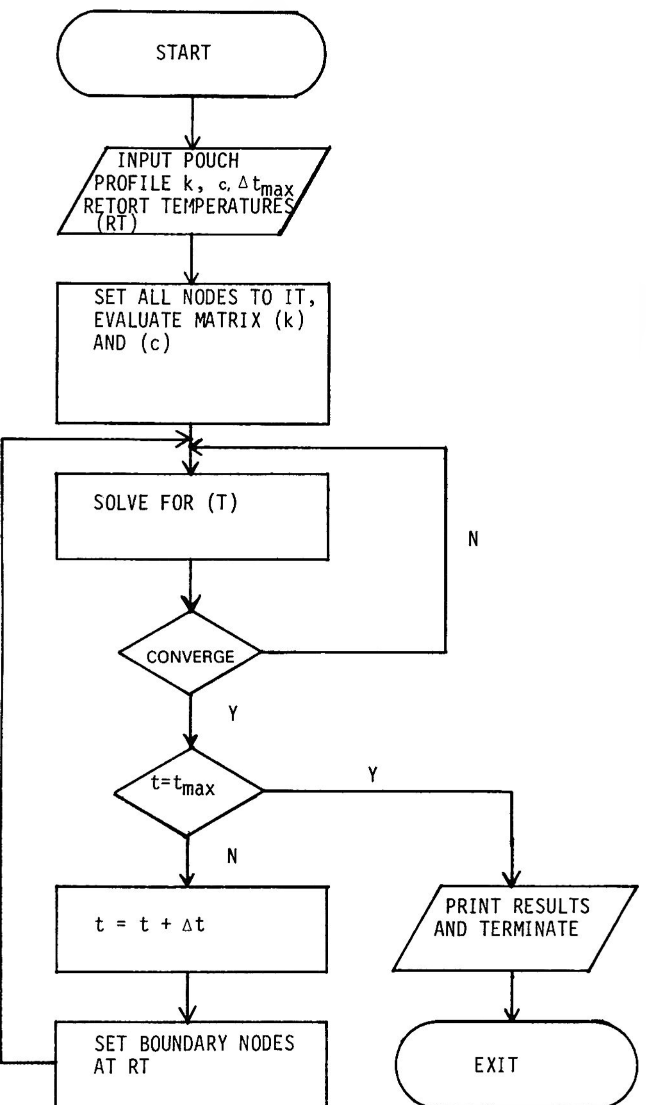

Fig. 5 — Abridged flow diagram for thermal process evaluation by the finite element technique.

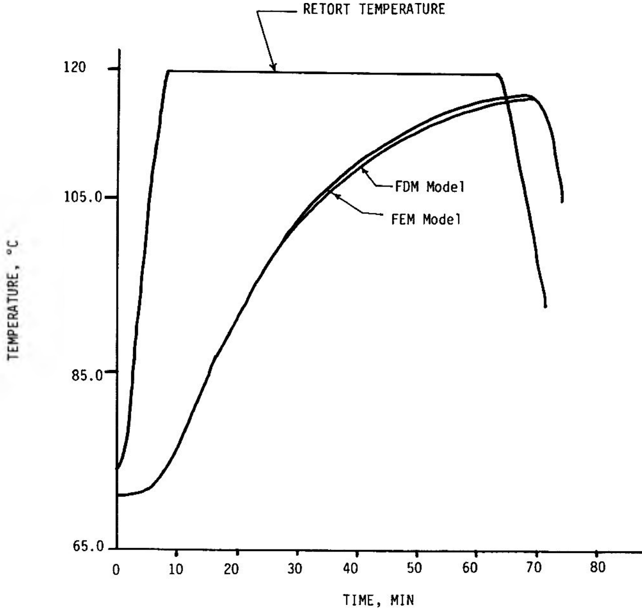

Fig. 6 — Temperature history at the coldest point in a 10% bentonite suspension as predicted by FDM and FEM models.

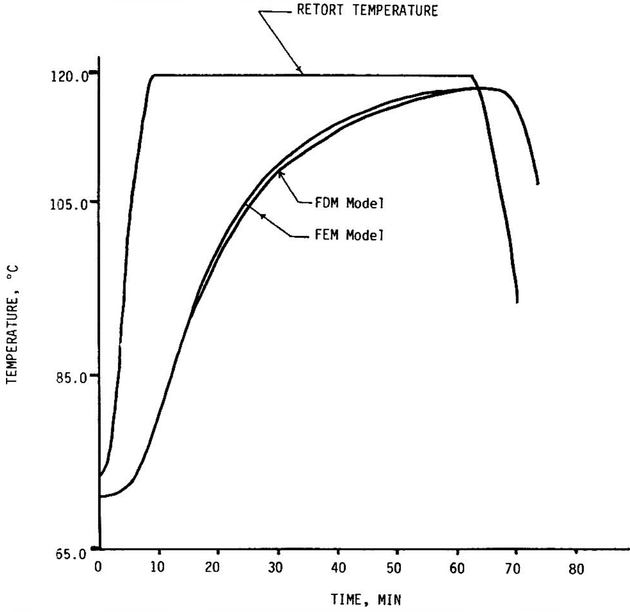

Fig. 7 — Temperature history at a side node in a 10% bentonite suspension as predicted by FDM and FEM models.

time increment during the process cycle, including come-up time and cool-down time. The holding time was determined by the total process time required to achieve a prefixed level of critical point sterilizing value (F_c) or a prefixed level of mass average sterilizing value (F_s).

The temperature estimated for each node point, at the end of each time step, was used to determine the concentration of survived target bacterial spore or nutrient by applying first order kinetics equation for inactivation of spores or destruction of nutrients, respectively, as shown below. (All symbols are defined in the Nomenclature.)

$$\frac{dc}{dt} = -\frac{C}{D} \qquad (6)$$

which integerates to:

$$C = C_o\ 10^{-t/D} \qquad (7)$$

The temperature dependence of D is expressed by:

$$D = D_r \cdot 10^{[(T_r - T)/z]} \qquad (8)$$

Table 2 — Comparison of nutrient retention, sterilizing values and spore destruction by the FDM and FEM computer programs

Parameters	FDM	FEM	% Difference
Process time (min)	69.00	69.00	none
Percent Thiamin Retention	50.19	48.92	2.59
F_c (min)	6.55	6.37	2.82
F_s (min)	7.91	8.07	1.98
% Reduction in spores	99.99	99.99	none

Table 3 — Major characteristics of FDM and FEM computer programs

Item compared	FDM Program	FEM Program
Type of technique used	Finite difference	Finite element
Computer language	Fortran IV	Fortan IV & others
Program capability	Heat transfer	Heat, stress & vibration analysis
Mathematical flexibility	(1) Used to study effects of any reaction whose kinetics is expressed as a function of time and temperature. (2) Iterates to find the process time required to attain a prefixed level of sterility at end of process.	Outputs only a time-temperature distribution within the body
Amount of input	One page for entire model	More than 4 pages
Computation time	Low	More than 4 times
Cost of transient run	Low	Very high

The critical point sterilizing value, F_c, at the end of each time step was estimated by:

$$F_c = \int_o^t 10^{[(T-T_r)/z]}dt \qquad (9)$$

where T is the temperature at the critical point or slowest heating point in the pouched product. The sterilizing value at the end of the process was estimated by summing up the lethality at the critical point for each time step using Simpson's (Carnahan et al., 1969) rule.

The mass average sterilizing value, F_s, was estimated using the following relationship:

$$F_s = D_r(\log C_o - \log C) \qquad (10)$$

The input process parameters were chosen in the range expected for most food processing applications (Manson et al., 1970) and are listed in Table 1.

MATERIALS & METHODS

COMPUTER SIMULATIONS were performed as follows: An institutional pouch (28.6 × 4.5 × 40.6 cm), filled with gelatin solution, was placed inside a cassette and allowed to set. The pouched gel was cut open and the boundary of the cross-section was determined using slide callipers. This cross-section was supplied to the program in terms of x-y coordinates. The program automatically superimposed an x-y grid pattern as shown in Fig. 1. In computer simulation, 10% bentonite suspension was used as the food simulant in a retortable pouch during thermal processing.

For each node point, at the boundary, proportionality constants A, B, C, and D were then determined. Eq. (3) and (4) were used to estimate temperatures at each node point for each time step for a given retort temperature. Finally, the sterilizing value F_c or F_s and the percent nutrient retention for given schedule process were determined. The computer flow diagram shown in Fig. 3, outlines the major steps involved in execution of the program.

Computer model using finite element technique (FEM)

A general purpose package computer program, ANSYS (Swanson Analysis Systems, Inc., PA), was used for transient heat transfer calculations. This program is based on classical engineering concepts and documented finite element technique. In this procedure, the input cross-section of the retort pouch was divided into isoparametric thermal elements with 2-dimensional conduction capability. The transient heat transfer analysis involved the use of a finite element technique in space and a finite difference technique in time.

The variable temperature in each element was approximated as a function of temperature values at the nodes (Segerlind, 1976):

$$T^e = T_1N_1 + T_2N_2 + T_3N_3 + \ldots + T_nN_n \qquad (11)$$

where T_1, T_2 represent nodal temperatures and N_1, N_2 are spatial shape functions derived from the geometry of the element.

The Fourier's heat conduction equation is expressed as:

$$[C_p]\{\dot{T}\} + [K]\{T\} = 0 \qquad (12)$$

Its recursive form is:

$$\left[\frac{C_p^e[C_p]}{\Delta t} + [K]\right]\{T_n\} = f\left[[C_p], \{T_{n-1}\}, \{T_{n-2}\}\right] \qquad (13)$$

where, [K] = thermal conductivity matrix; $[C_p]$ = specific heat matrix; $\{\dot{T}\}$ = derivative of temperature with time; Δt = increment in time; e = element number; n = current time level.

The temperature distribution within the pouched product approximated by a system of finite elements, may be obtained in a number of ways. This program used a method of 'weighted residuals' based on the minimization of residual (error) existing between the exact and approximate solution (Norrie and DeVries, 1978). The current value of the temperature at each node point was iteratively obtained using the nodal temperature from the two previous time steps by Eq. (13).

The largest cross-section of the pouch was supplied to the program in terms of x-y coordinates as in the case of the finite difference model. This cross-section was divided into isoparametric triangular elements (Fig. 4) using a subroutine built into the main program. The input process parameters and retort temperature profile used for this program were similar to those used for the finite difference model.

The time-temperature distribution obtained using the finite element technique was then used to estimate spore survivor and nutrient concentration at the end of each time step for each node point. The rest of the steps used to estimate total number of survivors, concentration of nutrients, F_c and F_s values were similar to those for the finite difference model. An abridged flow diagram shown in Fig. 5 outlines the major steps involved in execution of this program.

RESULTS & DISCUSSION

THE PROCESS PARAMETERS shown in Table 1 were used to run the computer programs developed in this study. The value of thermal diffusivity (α) of a 10% bentonite suspension shown in this table was experimentally determined using the method of Bhowmik and Hayakawa (1979). Other parameters were chosen in the range that are encountered in most cases of thermal process evaluation (Manson et al., 1970; Pflug and Odlaung, 1978).

The temperature history at the slowest heating point inside the retortable pouch filled with 10% bentonite suspension was predicted by the FDM (Finite Difference Model) and FEM (Finite Element Model) programs for 69 min of process time which included come-up, holding, and cool-down time. The results of this simulation are shown in Fig. 6. Next, the temperature histories at a side node predicted by the two models were compared (Fig. 7). In this case the thermocouple junction was positioned at an internal node point located at 1/5th the total width of the pouch (Fig. 1) along the mid-plane. To compare the two programs in terms of process evaluation, temperature distributions within the entire body of the pouch filled with 10% bentonite suspension were used to estimate destruction of target spores, nutrient retention and mass average ster-

ilizing value for a prefixed processing time. The results of this simulation are shown in Table 2.

A maximum temperature difference of 1°C and an absolute mean difference of 0.6°C were observed for the temperature histories predicted by the two models at the slowest heating point. This showed that the FDM model developed in this study could predict temperature inside a retortable pouch considering its actual shape as closely as predicted by the finite element model (FEM). Results in Table 2 indicate some differences in the values for nutrient retention and estimated mass average sterilizing values for 69 min of process time using 119°C as the retort temperature. This slight disagreement between the two sets of calculations could be attributed to built in truncation and round off errors and the difference in total number of elements used to divide the pouch in the two programs.

The major characteristics of the developed FDM and FEM models are shown in Table 3. The FEM model is based on a finite element technique and requires detailed inputs to run the program. The location of each node defining an element within the pouch needs to be specified. On the other hand, in the FDM model, the grid pattern is automatically generated using a built-in subroutine.

The FDM method is also extremely economical in terms of computer storage space and central processor time. In this method, all calculations could be accomplished with maximum central memory requirement of 3000 K-bytes, and central processor time of 16.0 sec on an IBM-3081 computer. It was observed that the FDM program reduced the computational cost at least by 40% as compared to the FEM model.

Overall, the FDM program is easier to execute and more economical in analysis of transient heat conduction in anomalous shaped retortable pouches as compared to the finite element technique (FEM model). The developed FDM model can be conveniently used for optimizing variable retort temperature profiles for thermal processing of retortable pouches.

CONCLUSION

THE COMPUTER MODEL (FDM) developed in this study closely emulated the effect of thermal processing on conduction heated foods packaged in anomalous shaped institutional size retortable pouches. The program has the capability to predict temperature distribution, estimate integrated lethal effect on target bacterial spores and degradation effect on any thermally vulnerable factors (nutrients) for a constant as well as for time varying retort temperature profiles.

This model extended the application of conventional finite difference technique to problems involving irregular shaped bodies in performing heat transfer analysis. Yet, the model maintained the inherent simplicity of the finite difference technique and the accuracy of other more complicated, time-consuming, finite element techniques. The modified finite difference technique developed in this investigation could be used to study heat and mass transfer in foods with irregular geometrical configuration.

APPENDIX

USING the Alternating Direction Implicit (ADI) method (Ames, 1977) to solve the Fourier heat conduction equation (Eq. 1), the following expressions are obtained:

Equation implicit in x-direction

$$\frac{T^*_{i,j} - T_{i,j,n}}{\Delta t/2} = \alpha T^*_{xx,i,j} + \alpha T_{yy,i,j,n} \qquad \text{(A-1)}$$

Corresponding equation in y-direction

$$\frac{T_{i,j,n+1} - T^*_{i,j}}{\Delta t/2} = \alpha T^*_{xx,i,j} + \alpha T_{yy,i,j,n+1} \qquad \text{(A-2)}$$

where T^* is the intermediate value of temperature at the end of the first half time step:

$$T_{xx} = \frac{\partial^2 T}{\partial x^2} \text{ and } T_{yy} = \frac{\partial^2 T}{\partial y^2}$$

The second order partial derivatives T_{xx} and T_{yy} can be further simplified using the Taylor series expansion (Carnahan et al., 1969). The body is first divided into rectangular elements of area Δx by Δy by superimposing a grid pattern (Fig. 1).

Now, expansion of Taylor series for $T_{i-1,j}$; $T_{i+1,j}$; $T_{i,j-1}$; and $T_{i,j+1}$ about the central value $T_{i,j}$ gives:

$$T_{i-1,j} = T_{i,j} - \Delta x T_x + \frac{\Delta x^2 T_{xx}}{2!} - \frac{\Delta x^3 T_{xxx}}{3!} + \text{---} \qquad \text{(A-3)}$$

$$T_{i+1,j} = T_{i,j} + \Delta x T_x + \frac{\Delta x^2 T_{xx}}{2!} + \frac{\Delta x^3 T_{xxx}}{3!} + \text{---} \qquad \text{(A-4)}$$

$$T_{i,j-1} = T_{i,j} - \Delta y T_y + \frac{\Delta y^2 T_{yy}}{2!} - \frac{\Delta y^3 T_{yyy}}{3!} + \text{---} \qquad \text{(A-5)}$$

$$T_{i,j+1} = T_{i,j} + \Delta y T_y + \frac{\Delta y^2 T_{yy}}{2!} + \frac{\Delta y^3 T_{yyy}}{3!} + \text{---} \qquad \text{(A-6)}$$

where,

$$T_x = \frac{\partial T}{\partial x},\ T_{xxx} = \frac{\partial^3 T}{\partial x^3},\ T_y = \frac{\partial T}{\partial y},\ T_{yyy} = \frac{\partial^3 T}{\partial y^3}$$

Rearranging:

$$T_x = \frac{T_{i+1,j} - T_{i-1,j}}{2\Delta x} + 0[\Delta x^2] \qquad \text{(A-7)}$$

$$T_{xx} = \frac{T_{i-1,j} - 2T_{i,j} + T_{i+1,j}}{\Delta x} + 0[\Delta x^2] \qquad \text{(A-8)}$$

$$T_y = \frac{T_{i,j+1} - T_{i,j-1}}{2\Delta y} + 0[\Delta y^2] \qquad \text{(A-9)}$$

$$T_{yy} = \frac{T_{i,j-1} - 2T_{i,j} + T_{i,j+1}}{\Delta y} + 0[\Delta y^2] \qquad \text{(A-10)}$$

in which,

$0(\Delta x^2)$ and $0[\Delta y^2]$ are error terms.

However, in situations where the boundary is irregular, the boundary may intersect the grid pattern at a location other than node as shown by Fig. 2. Then the distance between the node point under consideration and the boundary is some fraction of the distance defined for Δx and Δy. This fractional distance is expressed in terms of proportionality constants A, B, C and D whose value varies from 0 to 1 depending on where the boundary intersects the grid network. The effect of an irregular boundary on heat transfer is accounted for by rederiving the nodal temperature equations using the Taylor series expansion.

Based on this concept of an irregular boundary, equations A-3 through A-6 are modified as the following:

$$T_{i-1,j} = T_{i,j} - D\,\Delta x T_x + \frac{(D\,\Delta x)^2 T_{xx}}{2!} - \frac{(D\,\Delta x)^3 T_{xxx}}{3!} + \cdots \quad \text{(A-11)}$$

$$T_{i+1,j} = T_{i,j} + B\,\Delta x T_x + \frac{(B\,\Delta x)^2 T_{xx}}{2!} + \frac{(B\,\Delta x)^3 T_{xxx}}{3!} + \cdots \quad \text{(A-12)}$$

$$T_{i,j-1} = T_{i,j} - C\,\Delta y T_y + \frac{(C\,\Delta y)^2 T_{yy}}{2!} - \frac{(C\,\Delta y)^3 T_{yyy}}{3!} + \cdots \quad \text{(A-13)}$$

$$T_{i,j+1} = T_{i,j} + A\,\Delta y T_y + \frac{(A\,\Delta y)^2 T_{yy}}{2!} + \frac{(A\,\Delta y)^3 T_{yyy}}{3!} + \cdots \quad \text{(A-14)}$$

Simplifying, we obtained:

$$T_{xx} = \frac{BT_{i-1,j} - (B+D)T_{i,j} + DT_{i+1,j}}{BD(B+D)\,\Delta x^2/2} \quad \text{(A-16)}$$

$$T_{yy} = \frac{AT_{i,j-1} - (A+C)T_{i,j} + CT_{i,j+1}}{AC(A+C)\,\Delta y^2/2} \quad \text{(A-17)}$$

Substituting values of T_{xx} and T_{yy} in Eq. (A-2) and (A-3), we obtained Eq. (3) and (4) which represent the final form of the finite difference equations used in developing the computer model.

NOMENCLATURE

A,B,C,D	Proportionality constants
C	Concentration of spores (numbers)
C_o	Initial concentration of spore (numbers)
C_n	Concentration of nutrients (%)
C_p	Specific heat (J/Kg C°)
D	Decimal reduction time of spores (min)
D_r	Decimal reduction time at a reference temperature (min)
D_{nr}	Decimal reduction time for target nutrient (min)
e	Element number
F_c	Critical point sterilizing value (min)
F_s	Mass average sterilizing value (min)
K	Thermal conductivity of food (Watt/m C°)
t	Time (min)
T	Temperature (°C)
$\dot{T}$	Derivative of temperature with time
T*	Intermediate temperature (°C)
T_r	Retort temperature (°C)
z	Reciprocal of slope of thermal destruction curve of target microorganisms (C°)
α	Thermal diffusivity of food simulant (m^2/sec)

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Heat Transfer Study on Flame Pasteurization of Liquids in Aluminum Cans

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ABSTRACT

Heat transfer studies were performed in 209 × 413 aluminum cans using 1% bentonite suspension. The cans were heated in a pilot flame sterilizer. The temperature profile was practically uniform radially and axially after 4.5 min of heating. The slowest heating was experienced in the bottom (heel) of these cans. The temperature difference between the slowest heating point and the hot region over the flame decreased as the can was heated: from 15.6°C after 1 min to 6.7°C after 4.5 min of heating. The internal heat transfer coefficient at 40 rpm was measured and showed an increase with heating time.

INTRODUCTION

FLAME PROCESSING provides uniform and rapid heating, and it has been used as a high temperature-short time (HTST) process, as documented by Wu (1971), Klepetko and Longworth (1972), and Leonard et al. (1975a,b,c; 1976; 1977). A considerable amount of research has been done to model and to understand heat transfer to and within various containers when heated directly over a gas flame (Thomas, 1972; Paulus and Ojo, 1974; Fujiwara, 1975; Mejia, 1976; Merson et al., 1980; Peralta Rodriguez, 1982; Teixeira Neto, 1982; Peralta Rodriguez and Merson, 1982; 1983). Since its introduction (Cheftel and Beauvais, 1957; 1958), flame sterilization was applied as an HTST process and the subsequent research was done starting at 90°C, the minimum initial temperature at which containers of food should enter the flame heating section of the flame sterilizer. All research work, with the exceptions of Peralta Rodriguez (1982) and Peralta Rodriguez and Merson (1982; 1983), used the original French burner design where the combustible gas injected into the burner aspirated the air needed for combustion. Peralta Rodriguez (1982) and Peralta Rodriguez and Merson (1982; 1983) were first to use a custom designed version of the Flynn burner system (Flynn Burner Corp., New Rochelle, NY), which consisted of a rectangular, planar region of several ribbons of burners in parallel, in contrast to the single ribbon burner used in commercial (or experimental) flame sterilizers. The Flynn concept in burner design differs from the French in that with the Flynn system, stoichiometric combustion of gases can be achieved and, in comparative evaluations, the Flynn burner was at least 19% more efficient than the original French burner design (Heil, 1983).

The heating characteristics obtained in flame processing, i.e., speed of heating and uniformity of heat distribution, are desirable not only for sterilizing foods but also for in-container pasteurization of beverages. From previous research, it is known that burner type, flame intensity, product viscosity, agitation, headspace, can geometry, container-to-flame distance and can temperature are factors to be considered. Therefore, to consider implementation of flame heating for pasteurizing beverages, work was needed to evaluate any such untested conditions typical of the pasteurization process.

The objective of this work was to develop and evaluate internal can temperature profiles during flame heating in the 4.4°C (40°F) to 85°C (185°F) temperature range characteristic to pasteurization of some beverages and to determine internal heat transfer coefficients for a model liquid packed in nonsymmetrical, 209mm × 413mm aluminum beverage cans, using the Flynn burner system, designed for use in commercial flame sterilizers for canned products.

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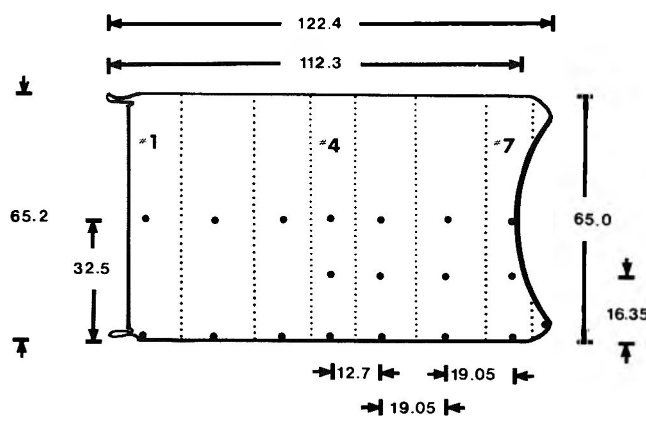

Fig. 1—Designated location of thermocouple positions in the 209 × 413 aluminum cans of 1% bentonite suspension, for determination of internal temperature profiles and internal heat transfer coefficients during flame pasteurization of the model liquid. On the center axis, thermocouples were at the center of individual elements. Element #1 was located near the lid whereas element #7 is located at the bottom of the aluminum can. Dimensions are given in millimeters.

METHODS & MATERIALS

Preparation of liquid models

One percent bentonite suspension was used as a model to determine temperature profiles. This concentration provided a suspension with a viscosity similar to that found in typical juices and nectars (Noh et al., 1985). The proper weight of bentonite (Pioneer Chemical Co., Los Angeles, CA), on dry basis, was added to distilled water. The settled clay was allowed to rehydrate for 4 hr, and then was agitated with a mechanical stirrer. To avoid the change in the heating rate between the first and subsequent heat processes reported by Jackson and Olson (1940), it was necessary to stabilize the suspension by heating it (sealed in 401 × 411 cans) for 2 hr at 121.1°C. The stabilized suspension could be used up to eight times without significant change in heating characteristics.

Preparation of cans

For measuring can temperatures radially and axially inside the cans, heat penetration rates were measured at each position indicated in Fig. 1. In each of six cans, four 30 gauge flexible copper constantan thermocouples (O.F. Ecklund Inc., Cape Coral, FL) were attached to support wires at selected positions using plastic connectors. The support structure (Fig. 2) was constructed from 0.1574 cm diameter stainless steel and was held stationary in the can to minimize any effect on convection patterns induced by heating and rotation of the can (Merson et al., 1980). One of the four flexible thermocouples was bent and fixed by tension to contact the inside surface of the can, centered over the flame (element #4). This thermocouple position was common to all cans and was used as reference in all runs. Two

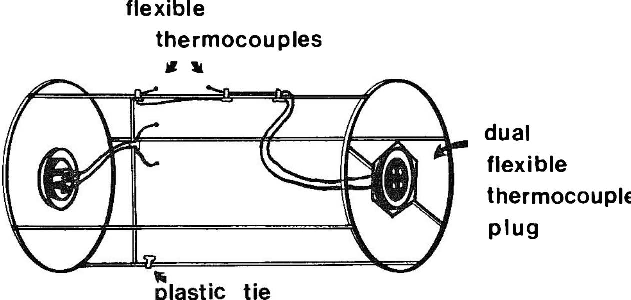

Fig. 2—Supporting structure for the thermocouples. Only four thermocouples could be installed in each can. One of the four thermocouples was always positioned on the can wall over the flame and was used as a reference in evaluating the reproducibility of heating conditions among different cans, thus data generated with different cans could be combined to generate the subsequent temperature profiles.

thermocouples through each end of the can were connected via dual rotary connectors (0. F. Ecklund, Cape Coral, FL) as shown by Noh et al. (1985) to a digital data logger (Model PD-2604, Esterline Angus Instrument Corp., Indianapolis, IN).

The 209 × 413 (65.2mm × 122.4mm) aluminum cans were filled with 368 ± 5g of 1% bentonite, allowing no headspace. After seaming, the cans were chilled to about 4.4°C in ice water, wiped dry, placed and rotated over the flame at 40 rpm, according to the recommendation of Leonard et al. (1977). The natural gas flow rate, unless indicated otherwise, was 6.43 L/min STP. The separation distance between the can and the burner was 1.5 cm, indicated as optimum by Peralta Rodriguez and Merson (1983). The burners used in this work were 0.95 cm wide and 44.5 cm long with 11 openings per cm (Flynn Burner Corp., New Rochelle, NY). Air and gas were stoichiometrically mixed by aspirating gas into a regulated stream of air. Flow of gas was measured with a flow meter (Model FP-1/4-10-G-10/4434396U10) with a carboloy float (Fisher & Porter Co., Warminster, PA).

Heating experiments

The digital data logger was programmed to monitor the temperature of the liquid every 10 sec. After confirming that the thermocouples in the can were functioning using a digital multimeter (Model 22-197 Radio Shack, Fort Worth, TX), the rollers of the flame sterilizer were started and adjusted to the desired can rotational speed. To avoid cold drafts during heat treatment, a 330 mm × 200 mm × 200 mm shield was built and placed over the can. The top of the shield was open to prevent heat accumulation. Ambient temperature measured near the ends of cans remained within 4°C from the start to the finish of heating.

The heating experiments were performed by placing the can on the rollers manually, igniting the burners, and allowing the contents of the can to heat from 4.4 ± 2.8°C up to 85°C. At the end of heating, the motion of the rollers and the burners were shut down, the can was removed from the burners and placed in an ice water bath to cool. The heating cycle was repeated to obtain three replications with each can. The can rotational speed was checked at least once during the heating cycle by measuring the time required for the can to complete 20-30 revolutions.

Heat transfer coefficient

Based on the positions (Fig. 1) where temperature data were obtained, the aluminum can was theoretically divided into seven disc-shaped elements by planes perpendicular to the can axis. It was assumed that the bulk temperature in each element was represented by the centerline temperature of each element.

For calculating the internal heat transfer coefficient, the centerline and the internal surface temperature profiles were used for each of the seven elements illustrated in Fig. 1. For calculating the inside heat transfer coefficient (from the can to the liquid), the film model suggested by Merson et al. (1980) was used. That is, the total amount of energy transferred (Q) was calculated using the experimental data, and Eq. (1):

$$Q = M\,Cp \cdot < \frac{dT}{d\theta} > \qquad (1)$$

Table 1—Experimental data for each element, used in the calculation of the internal heat transfer coefficient (h_i)

Element	$A_{(n)}$ (cm²)	dT/dθ (°C/sec)
1	28.59	0.32 ± 0.01
2	39.02	0.30 ± 0.01
3	32.51	0.30 ± 0.01
4	26.01	0.29 ± 0.02
5	32.51	0.31 ± 0.02
6	39.02	0.33 ± 0.01
7	29.13	0.32 ± 0.01
Avg.		0.31 ± 0.01

where M = mass of body heated, C_p = heat capacity of the body, and values of < dT/dθ > were obtained from the average slopes of the straight line portions of the heating curves for each element. Subsequently, the internal heat transfer coefficient, $<h_i>$, was calculated using an equation similar to that used by Merson et al. (1980), where

$$< h_i > = \frac{M\,C_p \dfrac{dT}{d\theta}}{\sum_{n=1}^{7} (T_{is(n)} - T_{b(n)})\,\pi\,D\,\Delta Z_{(n)}} \qquad (2)$$

In this equation, $T_{is(n)}$ = inside surface temperature; $T_{b(n)}$ = centerline or bulk temperature; $\pi D \Delta Z_{(n)}$ = area of each element; n = number or respective position of element. With aluminum beverage cans, only radial symmetry could be assumed, as the end configurations substantially differed (Fig. 1).

Statistics

Data were processed using applicable programs of MINITAB (Ryan et al., 1976).

Container integrity

Distortion of cans during heating was observed. After repeated (3) heating, the lithography (external) and enamel lining were observed for any sign of heat damage, such as, discoloration and/or blistering.

RESULTS & DISCUSSION

BENTONITE water suspensions have often been used to model food systems (Jackson and Olson, 1940; Paulus and Ojo, 1974; Merson et al., 1980; Unklesbay et al., 1980, 1981; Niekamp et al., 1984), when it was desirable to heat the material repeatedly without it suffering changes in heating properties, especially when preparation of cans with thermocouples and support wires was very complex. The heating properties of the stabilized 1% bentonite suspension were tested in terms of dT/dθ prior to the study and were found to be stable even after repeated heat treatments, as can be seen in the small standard deviations listed in Table 1.

Temperature profiles

Fig. 3 shows the temperature distribution along the inside surface of aluminum cans containing a 1% bentonite suspension. The data were plotted at 1 min intervals and represent the averaged temperatures of three replications. The internal surface temperature, initially between 3.3–5.6°C, reached an average temperature of 83.3–87.8°C in 4.5 min. The corresponding temperature at the bottom end corner or heel of the can reached an average temperature of 80.6°C. The bottom end corner or heel appeared to be the slowest heating region; however, as the heating time increased, the temperature difference between the center (over the flame) and the heel decreased from 15.6°C at 1 min, to 6.7°C at the end of 4.5 min of heating. Standard deviation for temperatures at the respective points ranged between ± 0.9°C and ± 6.6°C.

In the earlier stages of heating, there was a bell-shaped temperature profile on the internal surface of the can. This profile disappeared, however, after 4.5 min. At sterilization temperatures, Merson et al. (1980) showed a similar bell-shaped temperature profile which persisted even after 6 min of heating of a 303 × 406 can filled with 60% sucrose solution. The dis-

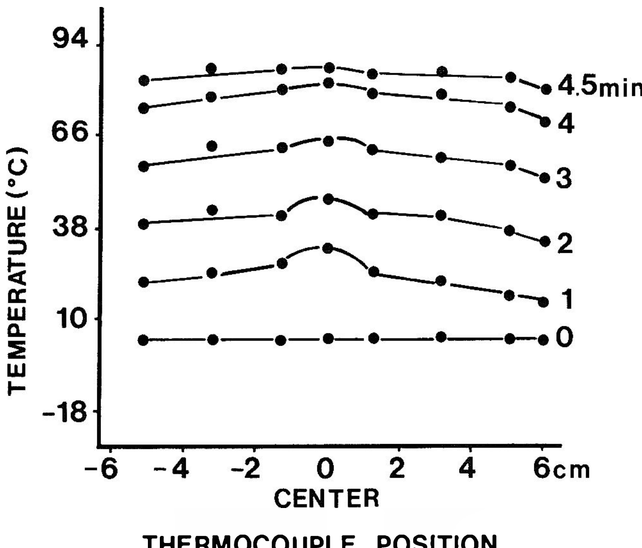

Fig. 3—Averaged internal surface temperature profile for 1% bentonite suspension in 209×413 aluminum can during flame heating at 40 rpm and 6.43 L/min gas flow rate.

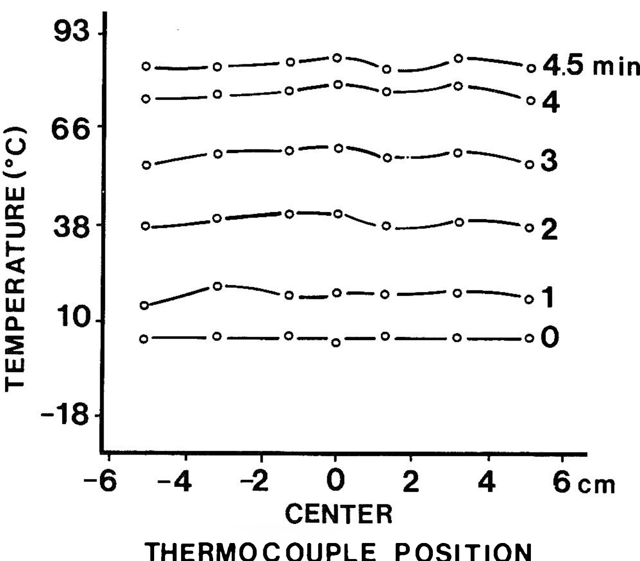

Fig. 4—Averaged centerline temperature profile for 1% bentonite suspension in 209×413 aluminum can during flame heating at 40 rpm using 6.43 L/min gas flow rate.

crepancies in these results may be attributed to differences in the medium and the heating rates used, and temperature ranges studied.

The range of values for internal surface temperatures at any point (Fig. 3) was greater than for any of the other positions. One reason may be that the thermocouples measuring the internal surface temperature of the aluminum can could not be welded to the aluminum can wall, so the flexible thermocouples were bent and held in position by the tension of the thermocouple wires. This contact between surface and thermocouples was not as positive as if welded, and may have added to the variation of temperatures measured. Use of epoxy to fix these thermocouples to the can wall was avoided because of the insulating effect of such bonding materials. The other reason that may have caused variations was that temperature measurements taken were variable with respect to the relative position of the fixed thermocouples as the cans were rotated over a stationary flame.

Initial temperature between replications also varied. The differences, however, decreased as heating time increased, which indicated that the rate of heat transfer was not seriously affected by the indicated initial temperature differences.

Unlike the cans in previous studies that were used for canning, aluminum cans were not symmetrical in shape, resulting in slightly asymmetric temperature profiles especially in the earlier stages of heating.

The axial centerline temperature profiles after 0, 1, 2, 3, 4, and 4.5 min, in Fig. 4, were fairly uniform. The maximum temperature difference between the center (in element #4) and the bottom end (element #7) of the can was 5.5°C. Standard deviation values for the temperatures at different points in the can ranged from ±0.1 to ±2.7°C.

Figure 5 shows the axial temperature distribution with the thermocouples located 1.625 cm from the can wall. There was only a 2.2°C difference between the temperature at the center plane (in element #4) of the can and the temperature near the bottom end (in element #7), after 4.5 min of heating. The standard deviation ranged between ±1.2°C and ±4.2°C. Similarly, Fig. 6 shows the radial temperature profiles for the thermocouples located at 1.27 cm from the center plane. A temperature difference of up to 12.6°C was observed after 1 min heating. This difference decreased to 0.8°C after 4.5 min of heating. Reproducibility of the heating rates above the flame, calculated from the straight portion of heating curve was 0.29±0.02°C/sec. According to work done by Merson et al.

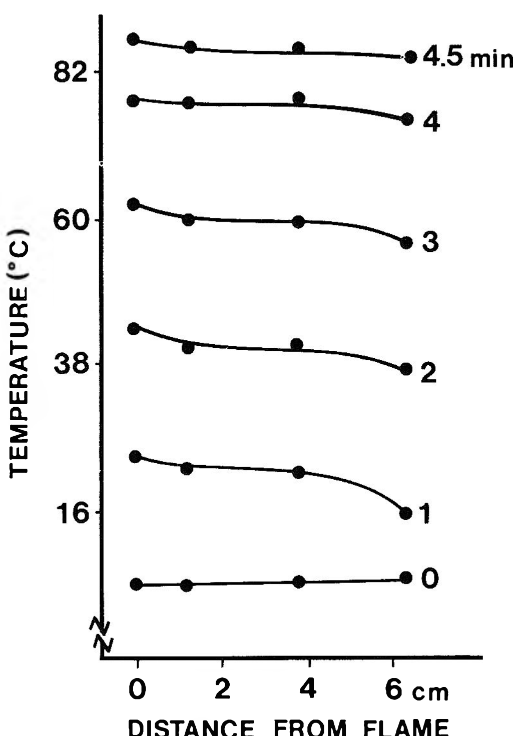

Fig. 5—Averaged axial temperature profile, 1.625 cm from the can wall, for 1% bentonite suspension in 209×413 aluminum can during flame heating at 40 rpm and 6.43 L/min gas flow rate.

(1980), a maximum temperature difference was expected at this position. Although the results reported herein are in agreement with the observations of Merson et al. (1980) in that the "hot spot" was not centered over the burner, one must not overlook the possibility that the phenomenon may also indicate whether optimum distance between the can and the flame has been achieved (Noh et al., 1985).

Internal heat transfer coefficient

Figures 3, 4, 5, and 6 showed that heat transfer was rapid and reasonably uniform. This would indicate that the heat dis-

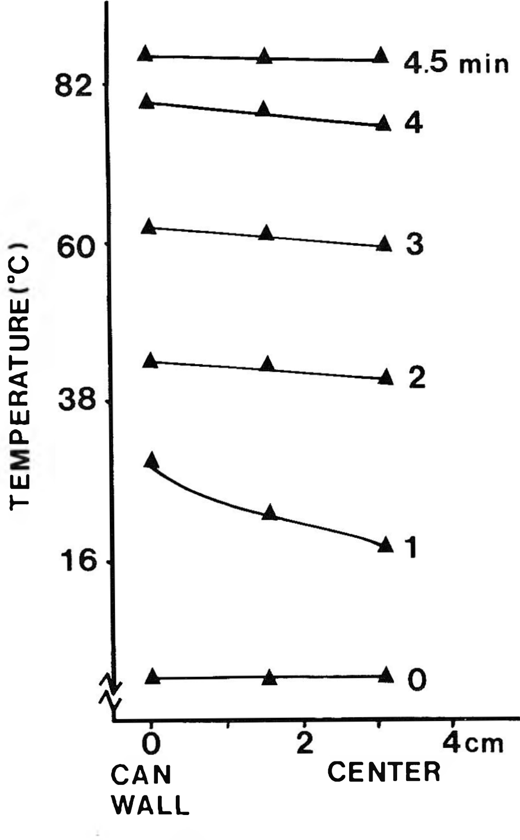

Fig. 6—Averaged Radial temperature profile for 1% bentonite suspension in 209×413 aluminum can flame heated at 40 rpm and 6.43 L/min gas flow rate.

Table 2—Temperature difference between inside surface temperature and centerline temperature ($T_{is} - T_b$)°C for each element, at indicated times of heating

Time (min)	Elements 1	2	3	4	5	6	7
0.5	7.4	10.4	16.1	15.9	9.4	8.8	4.8
1.0	8.6	6.3	12.6	14.5	6.8	4.6	1.7
1.5	4.6	3.7	2.9	7.1	3.2	6.2	1.0
2.0	3.4	4.4	2.7	8.4	5.6	3.6	0.7
2.5	2.3	5.4	4.1	6.8	7.7	1.9	2.5
3.0	2.5	4.6	3.0	4.1	3.8	2.0	2.4
3.5	0.9	3.2	4.4	5.8	5.1	0.6	0.9
4.0	0.4	2.3	3.4	4.2	4.1	1.3	1.3
4.5	0.6	3.1	1.1	1.3	1.9	1.0	1.8

tribution within the can was good under the conditions of the tests. The internal heat transfer coefficient for 1% bentonite suspension was calculated using Eq. (2), where M = 0.3665kg and Cp = 4.157 kJ/kgK (for 1% bentonite and 99% water). Relevant information on areas of individual elements, the corresponding values of dT/dθ and ($T_{is} - T_b$) are given in Tables 1 and 2. The results obtained with the assumption that the slope (dT/dθ) of the heating curve was constant are shown in Fig. 7. The h_i values ranged from less than 2,000 W/m^{2}K at the beginning of heating to over 12,000 W/m^{2}K after 4.5 min of heating.

Quast and Siozawa (1974) have shown data for the overall heat transfer coefficient for cans filled with sucrose solutions rotating in atmospheric steam. Their internal heat transfer coefficient value of 827.5 W/m^{2}K (J/m^{2}sK) was far less than any of the results of this work. However, since flame heating a less viscous liquid and no headspace were factors in this work, the results reported here need not be comparable to those reported by Quast and Siozawa (1974). Reported internal heat

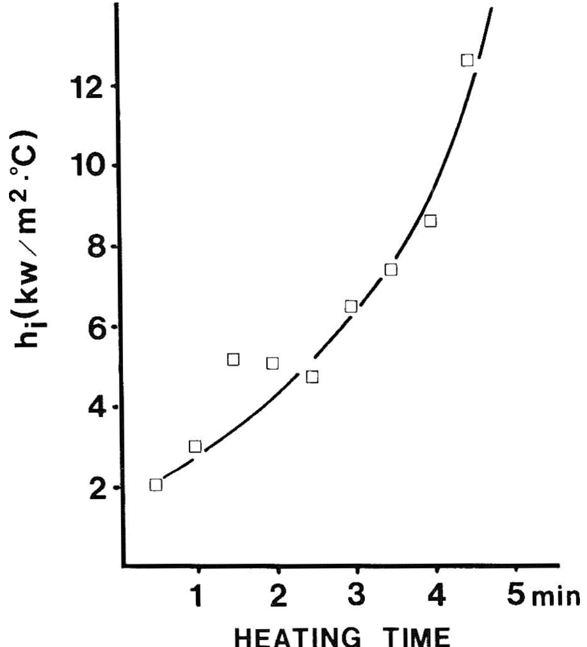

Fig. 7—Relationship of internal film heat transfer coefficients to time of heating for 1% bentonite in 209×413 aluminum can flame heated at 40 rpm and 6.43 L/min gas flow rate.

transfer coefficient (h_i) values for flame heating various liquids models ranged from 269.2 to 621.7 J/m^{2}sK (Peralta Rodriguez and Merson, 1982) and 437 to 500 W/m^{2}K (Merson et al., 1980), each using different burner systems, media more viscous than 1% bentonite and higher working temperatures than reported in this work. The use of different burner systems and liquid models, each, could account for differences in the h_i values obtained. The closest h value is given by Toledo (1980) who showed that h on the water side of a heat exchanger was 1925 J/m^{2}sK, when assuming a turbulent flow, which minimized the resistance to heat transfer. The lowest h_i = 1999 W/m^{2}K value found in this work reasonably agrees with Toledo's value. Agitation (rotation) of the cans over the flame is known to induce heat transfer and distribution in the can (Leonard et al., 1977) and would diminish resistance to heat transfer, as experienced by Toledo (1980). The observations of high h_i values in flame heating are also supported by the temperature distributions shown in Fig. 3, 4, 5, and 6 where rapid achievement of uniform temperatures was realized. This could be credited, in addition to can rotation (forced convection), to the relatively low viscosity of 1% bentonite, as compared to the high viscosity sucrose solutions used by others. The apparent increase in h_i values with time of heating (Fig. 7), however, is important. As Fig. 8 indicates, the heating rates measured at both the center and wall of the can converge with time over the flame, decreasing ($T_{is} - T_b$). On heating, the viscosity of the bentonite solution would also decrease with rising temperature and facilitate convective heating, especially since the container was agitated, resulting in the achievement of uniform temperature distribution within the container. Thus, the combination of changes under the reported experimental conditions resulted in measurable increase in h_i, to the extent that the external heat transfer coefficient (h_o) became limiting to the uptake of heat, i.e., as $1/h_i$ approached zero, the overall heat transfer coefficient U approached the value of h_o, as shown by Toledo (1980). Data showing the reported increase in h_i has not been demonstrated, whereas the importance of h_o in flame heating is well documented. Some of the h_o values reported only range between 28–57 J/m^{2}sK (Fujiwara, 1975) and set at 26.38 J/m^{2}sK (Peralta Rodriguez and Merson, 1982). While unavoidable errors in this work for measuring (T_{is} − T_b) and assuming that dT/dθ was constant could affect the

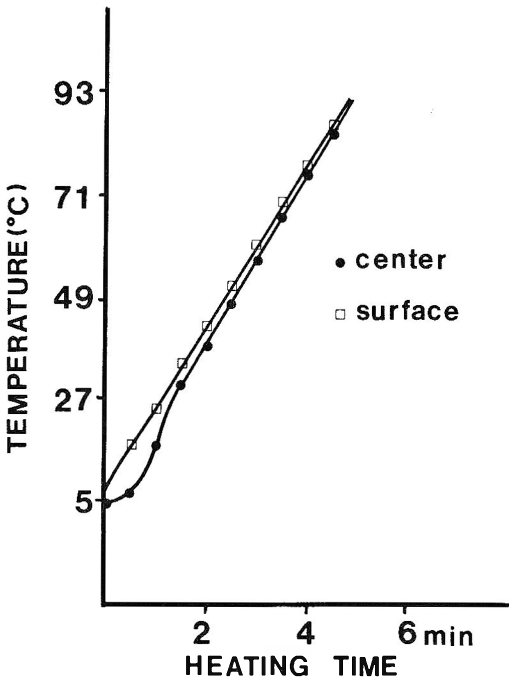

Fig. 8—Comparison of centerline and inside surface temperatures over the flame for 1% bentonite suspension in 209×413 aluminum can flame heated at 40 rpm and 6.43 L/min gas flow rate.

absolute h_i values, the relative changes observed remain valid, especially after the first minute of heating, past the lag period shown in Fig. 8. This lag was contributed to lack of headspace, the higher initial viscosity of the bentonite solution and the condensed water, a by-product of combustion, on the chilled cans observed at the beginning of heating. The insulating effect of the condensate and the heat required to evaporate it could decrease the rate of heat transfer to the contents, although, considering that the energy needed for evaporation of a small amount of condensed water (0.5 to 0.7g) was only 16J/sec, except for its insulating properties, evaporation of condensate should not be considered a serious influence on the total heat transfer. Reduction of the lag phase will be realized in commercial application by allowing some headspace (Leonard et al,. 1977). However, in this work, it was important to show results associated with the most difficult condition, such as no headspace, which hindered heat distribution in the aluminum can.

Container integrity

Except for the normal expansion of the top end of the aluminum cans during heating, no distortions of the containers were observed. Neither the lithography on the cans, nor the lining showed any indication of heat damage, even after repeated (3) heating of the containers.

CONCLUSIONS

EXPERIMENTS with a model system which simulated juices and nectars indicated that the flame pasteurization temperature distribution was uniform in aluminum cans. Based on temperature measurements at the can inside surface and can center and the slowest heating heel of the aluminum can, the maximum temperature difference did not exceed 6.7°C after 4.5 min heating to pasteurization temperature (85°C). This would indicate that instantaneous boiling which could damage heat sensitive components in a beverage should not occur during the flame pasteurization treatment of such products.

In the temperature range examined (4.4 – 85°C), the aluminum cans filled with 1% bentonite suspension withstood flame pasteurization without any visible damage to the structural integrity of the cans, to external lithography or to the internal enamel lining.

Internal heat transfer coefficients calculated show that the outside heat transfer coefficient became limiting to the uptake of heat in flame processing. The high internal heat transfer coefficients and thermal conductivity of the aluminum can, with induced convection, facilitated the rapid achievement of uniform temperatures in the cans.

Flame pasteurization of beverages appeared to be feasible.

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Hindrance of Hemicellulose and Cellulose Hydrolysis by Pectic Substances

NOACH BEN-SHALOM

ABSTRACT

Treating the tissue of the grapefruit segment membrane with pectinase and cellulase decreased the content of xylose and glucose significantly more than the sum of the separate effects. The synergistic effect obtained by the combination of pectinase and cellulase showed that the pectic substance sterically masked the hemicellulose and cellulose. Breaking down the barrier of the pectic substance with pectinase allowed a significant increase in the hydrolysis of the hemicellulose and cellulose. Preparation of alchol-insoluble solids from fresh tissue modified the structure of the pectin in the cell wall and prevented its steric hindrance to breakdown of hemicellulose and cellulase. Extraction of pectic substances from the tissue by NaOH greatly increased cellulose hydrolysis by cellulase.

INTRODUCTION

THE GENERAL CELL WALL MODEL proposed by Preston (1974) in which the cellulose microfibrils are coated with hemicellulosic polymers and are separated by pectic polysaccharides, is still accepted Taiz (1984). According to Preston (1974), the matrix of the cell wall owes its mechanical properties to the combined effect of a few strong bonds and many small groups of weak bonds. The links between the polyuronides are weak; those between the pelyuronides and hemicelluloses are also weak, but between the hemicellulose and the cellulose the links are strong. Another school of thought is based on the view that the cell wall matrix is formed by covalent bonds. These include glycosidic linkages between sugar residues, between arabinose and hydroxy proline residues of the wall protein Lamport (1980), or between ferulic acid residues (Fry, 1982; Smith and Hartely, 1983). We still do not have good evidence that shows that the pectic polysaccharides sterically mask the hemicellulose and cellulose in the cell wall. The purpose of this study is to use cellulase and pectinase as a tool to show the steric hindrance of hemicellulose and cellulose hydrolysis by the pectic substances.

MATERIALS & METHODS

MEMBRANES of grapefruit segments were prepared from *Citrus paradisi* Macfadyan cv. Marsh seedless. The membranes of the segments were peeled and washed several times with distilled water to wash out the soluble sugars, then lyophilized. The dry membranes were ground into powder and stored at − 18°C (Fig 1). This powder was found to be a homogenous raw material which gave reproducible results. Alcohol-insoluble solids were prepared from the fresh tissue by homogenizing 20 grams of the membrane three times with 100 mL 70% ethanol. Finally, the tissue was dried with 96% ethanol. The enzymatic activity was studied in a reaction mixture which contained 25 mg segment membrane powder and 3 mg commercial cellulase (Maxazyme c-200 from Gist Brocades) or 3 mg commercial pectinase (c-80 from Gist Brocades) in 10 ml buffer (sodium acetate 0.1M at pH 4.5). The mixtures were incubated for 40 min at 45°C. The reaction was stopped by heating the mixture for 5 min at 85°C. The pellet was collected after centrifugation for 5 min at 12.000 x *g*,

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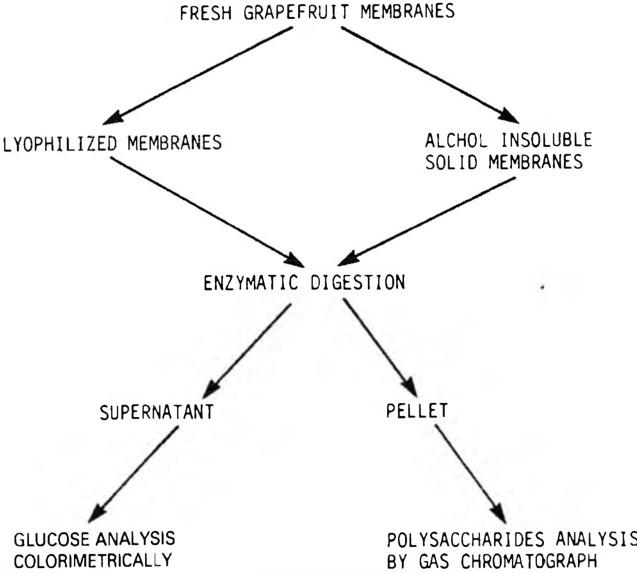

Fig 1—Scheme of the treatments for analysis the enzymatic degradation of the polysaccharides in the grapefruit membrane tissue.

rewashed with the buffer, lyophylized, and stored at − 18°C. The composition of the polysaccharides which remained in the pellet was analyzed by gas chromatography (Sloneker, 1972). The polysaccharides were hydrolyzed to monomers by sulfuric acid and subsequently determined by gas chromatography as alditol acetate. Uronic acids in the hydrolyzates were determined colorimetrically as described by Blumenkrantz and Asboe-Hansen (1973). Pretreatment of the tissue with HCl was done using 0.1N HCl for 10 min at 25°C. Then the acid tissue was washed with distilled water and 0.1M buffer sodium acetate at pH 4.5 was added with the appropriate enzyme. After incubation of the enzymes for 40 min at 45°C, as described previously, the amount of glucose in the aqueous extract was determined. The pectic substances were extracted four times from the cell wall in a system which contained 0.2 g tissue, 0.5N NaOH and 0.2% EDTA at 25°C. The amount of glucose found in the aqueous extract after cellulase activity was determined by glucose oxidase and peroxidase, using Sigma diagnostic unit No. 510.

RESULTS & DISCUSSION

PRELIMINARY EXPERIMENTS in our laboratory on maceration of various citrus plant tissues showed that when pectinase and cellulase were used together there was always a synergistic effect compared with their effects separately. To study this effect further, we used the alcohol insoluble membranes of grapefruit tissue (Fig. 1). The polysaccharides present in this tissue consist of ca 45% pectic substances. To check the possibilities that the pectic substances can mask the hemicellulose and cellulose and partially prevent their enzymatic hydrolysis, we treated the tissue with commercial cellulase (which contained hemicellulase), with or without pectinase. The residual polysaccharides which remained in the pellet after enzymatic hydrolysis of the tissue are shown in Fig. 2 The main neutral sugar in the nontreated tissue was glucose, followed by arabinose and xylose. Mannose and rhamnose made

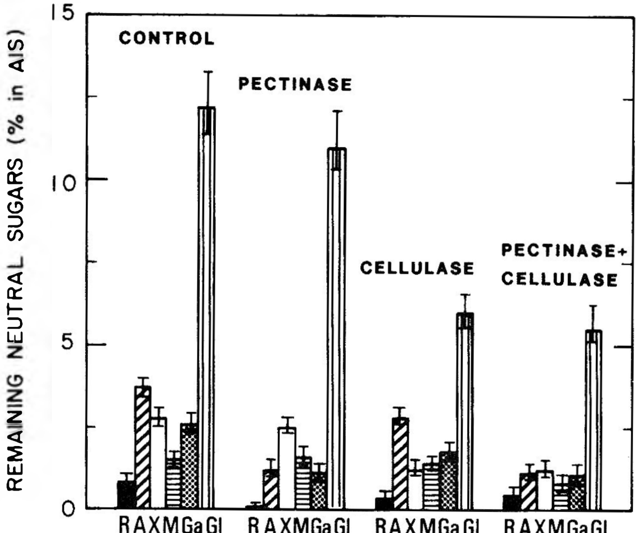

Fig 2—Degradation the alcohol insoluble solids of the grapefruit segments membrane with pectinase and cellulase. R: rhamnose; A: arabinose; x: xylose; M: mannose; G: galactose; Gl: glucose.

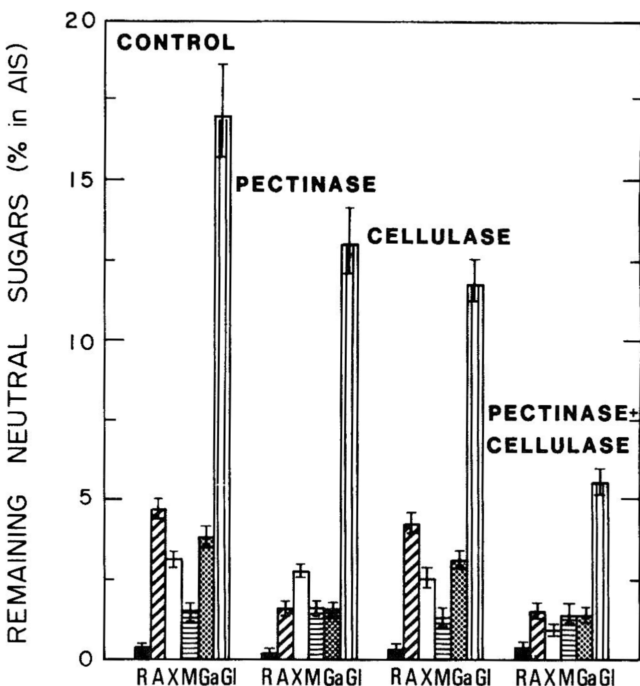

Fig 3—Degradation the cell wall of the fresh grapefruit segment membrane by pectinase and cellulase. R: rhamnose; A; arabinose; x: xylose; M: mannose; G: galactose; Gl: glucose.

up less than 1.5%. Addition of pectinase to the tissue reduced the amount of xylose by 11% and the amount of glucose by 23%. Xylose is a good indicator for hemicellulose and glucose for cellulose (McNeil et al. 1984). Hemicellulose and cellulose are closely connected and, according to the model proposed by Preston (1974), the cellulose microfibrils are coated with hemecellulose polymers. Part of the decrease of xylose and glucose by pectinase was due to the residual activity of cellulase and hemicellulase which exist in the commercial enzyme. Arabinose and galactose were the main neutral sugars that decreased after pectinase activity. This can be explained as due to their existence in the cell wall as side chains of the polyuronides (McNeil et al., 1984) and due to the existence of arabinases and galactanases in this commercial product. Addition of commercial cellulase to the tissue reduced the xylose content of the residual cell wall by 19% and the glucose content by 21%. In this treatment the amount of arabinose and galactose changed very little compared with the concentrations in nontreated tissue. Addition of pectinase and cellulase together decreased the level of xylose by 69% and that of glucose by 68%. In this case, the breakdown caused by pectinase and cellulase together was greater than the sum of their effects separately. This synergistic effect confirms our assumption that cellulose and hemicellulose in the cell wall are sterically masked by the pectic substances. Thus, the main effect of pectinase is to hydrolyze the pectic polysaccharides and to expose the hemicellulose and cellulose to enzymatic degradation. In trying to prevent the steric hindrance of hemicellulose and cellulose by the pectic substances, alcohol-insoluble solids (A.I.S.) were prepared from the fresh tissue (Fig 1). In the nontreated tissue, xylose and galctose were *ca* 10% lower than in the tissue which was washed with distilled water; arabinose was 27% lower and glucose 28% lower (Fig. 2). One of the explanations for the difference in the amount of neutral sugars between the two treatments is the existence of some oligosaccharides in the cell wall which were not precipitated by ethanol, but still existed in the tissue after it was washed with water. Based on this, we assume that part of the degradation effect related to the pectinase and cellulase (Fig. 2) was due to partial extraction of the oligosaccharides from the tissue during preparation of the A.I.S. from it. Addition of pectinase to the A.I.S. reduced the amount of xylose and glucose by 20% (Fig. 3). Addition of cellulase reduced the concentration of xylose by 56% and glucose by 51%. The combination of pectinase and cellulase reduced the level of xylose (56%) and glucose (55%), to almost the level of cellulase alone. The larger concentration of xylose and glucose released from the alcohol treated tissue, and the fact that addition of pectinase did not increase the hydrolysis of hemicellulose and cellulose, indicated that there was no stearic hinderance of hemicellulose or cellulose by the pectic substances. According to Jarvis (1982), two classes of uronic acids existed in the cell wall, convalent and nonconvalent-bound. Studies of sycamore suspension cell wall by Darvill et al. (1980) showed that the neutral and acidic pectin polysaccharides were covalently attached to the hemicellulose. It is possible that during dehydration of the tissue by ethanol, breakdown of noncovalent bonds took place in the cell wall and especially in the pectic substances, which tend to be highly hydrated and weakly bound polymers (Van Buren, 1979). As a result of this effect strong steric changes took place in their structure and the pectic polysaccharides no longer masked the hemicellulose and cellulose and prevented their enzymatic degradation. In an attempt to change slightly the pectic substances in the cell wall by gentle chemical treatment, we dipped the tissue in 1% HCl at 25°C for 10 min. Pretreatment with HCl before addition of pectinase did not release more glucose from the tissue (Table 1). Pretreatment of the tissue with HCl, followed by addition of cellulase, increased by 50% the amount of glucose which was released from the tissue. A combination of cellulase and pectinase increased the release of glucose by

—*Continued on page 730*

Table 1—Effect of pretreatment of the tissue with HCl, pectinase or extraction of the pectic substances with NaOH and EDTA on the concentration of the break-down products of cellulase in the aqueous extract

Treatment	Glucose mg/g dry weight tissue
Control	0.8
Control + HCl	0.8
Pectinase	3.0
Pectinase + HCl	2.8
Cellulase	4.0
Cellulase + HCl	6.0
NaOH + EDTA	3.0
Pectinase + cellulase	26.1
NaOH + EDTA + cellulase	43.7

Study of the Degradation Products Formed During Extrusion Lamination of an Ionomer

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ABSTRACT

Headspace concentration techniques were used to tentatively identify the volatile products formed during the extrusion process of thermoplastic polymers to produce ionomers which have many applications as food packaging materials. Compounds representing alcohols, aldehydes, ketones, and hydrocarbons were tentatively identified. Time/temperature studies showed that the quantity of compounds formed depended on the conditions used for resin processing. The rate of release of compounds was also found to be temperature dependent. Sensory evaluation data confirmed the instrumental findings.

INTRODUCTION

IONOMERS are defined as linear organic polymers which are copolymerized with a minor proportion of an acid function which is neutralized to varying degrees by a metal or quaternary ammonium ion (Longworth, 1984). This class of compounds is of great interest to the plastic industry because some constitute a class of thermoplastic materials that offer the solid state properties of crosslinked hydrocarbon polymers and the melt-flow properties of uncrosslinked hydrocarbon polymers.

Ionomers in the form of films or laminates exhibit the following properties: toughness, clarity, oil resistance, high melt strength, and lower temperature sealability. This combination of properties meets the requirements of flexible food packaging so well that it is the largest application for ionomer resins. Vacuum packaging of processed meat has become one of the most outstanding areas for application of ionomers. Soups, seasonings and spices are examples of products which use ionomer seal layers in form-fill-seal packaging construction. Other applications for ionomers are now developed from the coextrusion process. This process allows the use of ionomers in structures with beneficial properties without the need for adhesive laminations resulting in considerable cost reduction and freedom from solvent problems. The manufacture of packaging laminates with extrusion coated layers from ionomer resins is often conducted under conditions of high temperatures. These conditions can induce thermo-oxidative reaction with the formation of volatile compounds.

No reference on the thermostability of ethylene ionomers was found in the literature. Since polyethylene is the major component of this polymer, the hypothesis was formed that similarities existed in the mechanisms of degradation reaction of both thermoplastics. A free radical chain mechanism has generally been accepted to explain the thermal oxidation of polyethylene (Hoff and Jacobsson, 1981).

Headspace analysis by gas chromatography is the most often used technique to analyze the compounds that can migrate from the packaging material (Wilks and Gilbert, 1968). Because only the more abundant and more volatile compounds of thermal degradation reactions exist at detectable levels by direct headspace analysis of the packaging material samples, headspace concentration has to be used before gas chromatography/mass spectrometric analysis. Dynamic procedures for the preconcentration of headspace volatiles have been developed in recent years. Physical sorption on a polymeric sorbent is the commonly used procedure covering different applications such as analysis of flavors in food and beverages (Buckholz et al. 1980; Charalambous, 1978; Singleton and Patee, 1980), determination of residues in polymers (Westendorf and Grote, 1983), and analysis of residual solvents in laminates (Eiceman and Karasek, 1981; Wilks and Gilbert, 1968, 1972). Tenax GC, Poly(2,6-diphenyl-p-phenylene oxide), is the most widely used organic polymeric absorbent for the pre-concentration of trace organic volatiles in different media because of its high thermal stability (up to 450°C) despite its limited specific surface area (19–30 m/g) (Sakodinskii and Kiimskoya, 1974).

There are many studies in the literature where statistical techniques have been applied to correlate sensory and instrumental evaluation of flavor in food products (Clark and Nursten, 1977; Dravnieks et al., 1973; Powers and Quinlan, 1974; Walking, 1982). Only one reference was found (Fales and Stover, 1983) where the odor of resins used for packaging was tentatively correlated with its chromatographic analysis. Covariance or regression analysis is usually most appropriate and fruitful to establish general relations between sensory and objective data (Powers, 1982).

In some of the ionomer extruded laminates, not only was an adverse odor easily detected by sensory evaluation, but also migration to package contents was observed in preliminary tests. Therefore, the objectives of this study were: (1) to identify the volatile products formed during the film manufacturing process with the ionomer resin; (2) to correlate the extrusion coating process conditions with the rate of formation of volatile compounds; and (3) to correlate instrumental and sensory evaluation data.

MATERIALS & METHODS

SURLYN IONOMER RESIN in form of pellets and aluminum foil laminates were obtained from E.I. Du Pont De Nemours & Co., (Wilmington, DE). The ionomer was extruded on the aluminum foil at three different temperatures and were identified as follows: (1) Laminate A was extrusion coated at 282°C; (2) Laminate B was extrusion coated at 304°C; (3) Laminate C was extrusion coated at 324°C. These temperatures represent the upper range of extrusion conditions which have been implicated as possible causes of adverse flavor effects in foods packaged in contact with the extruded polymer.

Sample preparation

Laminates. Forty grams of ionomer laminate were cut in small pieces (2.5 cm × 1 cm) and placed into a 500 mL jar. This jar was closed with a special screw cap provided with inlet and outlet for flushing with h:gh purity helium. The closed jar, placed inside an oven, was heated at 100°C for 90 min, while helium was flushed through at a flow rate of 25 mL/min. The headspace was also collected

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at 150°C for 90 min. The compounds released from the material to the headspace were absorbed in a small stainless steel trap (15 cm × 0.6 cm) containing 450 mg of Tenax GC (Supelco, Inc.) kept in a dry ice bath. The temperature of 150°C was selected after a time/temperature study of volatile release showed that no new compounds were formed at this temperature compared to those at lower ones and represented a maximum release with time.

Resins. Six grams of resin in the form of pellets were mixed with diatomaceous earth in an aluminum dish that was placed into the 500 mL jar described above. The procedure used was the same as described for laminates.

GC/FID Analysis

After concentration of volatiles, the trap was inserted into a heating unit connected with the injection port of the GC. The heating unit was kept at 250°C. The GC was provided with a splitter before the FID detector. This allowed the sample to be sniffed while it was analyzed. Helium was used as carrier gas. The GC column was kept at 25°C during the desorption process that took 30 min. The column temperature was programmed from 50 to 200°C at 4°C/min. The columns used were: (1) 80/100 Supelcoport coated with 5% SE 30, stainless steel 25.7 cm × 0.32 cm; (2) 60/80 Supelcoport coated with 10% OV351, stainless steel 30.8 cm × 0.32 cm; (3) 80/120 Carbopack B. 3% SP1500, stainless steel 2.57 cm × 0.32 cm.

GC/MS Analysis

The same procedure used for GC/FID analysis was used here. The instrument used was Hewlett Packard 5990A GC/MS. Columns used during the analysis were the same as for GC/FID analysis. Helium was used as a carrier gas at a flow rate of 25 mL/min. Oven temperature was programmed to start at 50°C and to end at 200°C at a rate of 4°C increase. Injection port temperature was set at 200°C. With the peak finder mode, the mass spectra of each peak was obtained and manually compared to data in the literature for tentative identification.

Sensory evaluation

High quality natural spring water from glass bottles was packed in pouches made of the test aluminum foil laminates from laminates A, B and C and stored at 40°C for 24 hr. The sensory evaluation was carried out using the test — Rating Difference/Scalar Difference from Control (IFT, 1981). A panel of 33 experienced judges was used to taste the samples. Judges received all samples simultaneously, and identified control, coded experimental treatments, and a hidden control. A numerical scale was used ranging from: 1-No difference from the control, to 9-Extreme difference from the control. The control was the same spring water, stored in glass bottles under the same conditions as the samples.

Statistical analysis

Analysis of variance was applied to establish the statistical significance of the difference determined with the sensory test. The least significant difference (LSD) between panel scores was used to ascertain differences between scores (Kramer et al., 1982).

Multiple Regression Analysis was run to find a correlation between the sensory and instrumental results obtained. The panel scores were the dependent variable and the areas of six selected peaks of the GC/FID chromatograms were the independent variables. SAS (1979) was the computer program used for all statistical procedures.

RESULTS & DISCUSSION

Identification of compounds formed during the degradation reaction

All the analyses of identification of compounds were done using 150°C as the headspace collection temperature. Although the GC/FID chromatograms obtained from samples collected at 100°C and 150°C differed only on the height of the peaks,

Table 1—Compounds tentatively identified in the resins and laminates

Resin		Laminates
SP 1500	OV 351 & SE 30	SP 1500
Aldehydes	Alcohols	Aldehydes
2-methyl butanal	2-ethyl-1-hexanol	butanal (1)
pentanal	2-propyn-1-ol	2-methyl-2-propenal (4)
hexanal	4-tert-butylcyclohexanol	2-methyl-2-butenal (7)
3-methyl hexanal	7-ethyl-2-methyl-4-undecanol	2-methyl butanal (6)
heptanal		pentanal (9)
		hexanal (17)
Alcohols	Unsaturated hydrocarbons	heptanal (25)
		2,4-dimethylpentanal (22)
2-methyl-1-propanaol	1-pentadecene	acetaldehyde (A)
1-butanol	2-methyl-1-octene	
1-nonanol	5-butyl-4-nonene	Alcohols
Isooctyl alchol		
2,2,4-trimethyl-1-pentanol	Saturated Hydrocarbons	ethanol (A)
cyclohexanol		2-methyl-1-propanol (A)
ethanol	3-methylpentane	2-butanol (C)
	2, 2-dimethyl pentane	1-butanol (5)
	3-ethylpentane	1-pentanol (12)
Ketones	2,2,3-trimethylpentane	
	2,2,3,3-tetramethylbutane	Ketones
2-butanone	3,4-dimethylhexane	
2-pentanone	2,2,4,4-tetramethylpentane	2-butanone (2)
3-methyl-2-pentanone	2,2,4-trimethylhexane	2-pentanone (8)
3-heptanone	2,2,5-trimethylhexane	4-methyl-2-pentanone (13)
5-methyl-2-heptanone	2,2,4-trimethylheptane	3-hexanone (15)
3-heptanone	2-methyl-5-ethylheptane	2-hexanone (16)
	Tert-butylcyclohexane	3-heptanone (23)
Aromatic Hydrocarbons	2,2,4,6,6-pentamethylheptane	2-heptanone (22)
	5-methyl-5-ethyldecane	
toluene	2,5-dimethyltridecane	Aromatic Hydrocarbons
	4,11-dimethyltetradecane	
	2,6,10-trimethyltridecane	toluene (18)
Unsaturated Hydrocarbons	2-methylhexadecane	
	4,9-dipropyldecane	
3-methylpentene	2,6,10,14-tetramethylpentadecane	Unsaturated Hydrocarbons
4-methylpentene	4-propylheptadecane	
2-methyl-2-butene	4-methylnonadecane	2-methyl-2-butene (B)
1-pentene		
Saturated Hydrocarbons		Saturated Hydrocarbons
isopentane		isopentane (3)
2,2,3,3-tetramethylbutane		hexane (10)
		2,5-dimethylheptane (26)

* The identification in parentheses corresponds to the peak from the GC/FID profile of Fig. 1, with the letter designating compounds of Table 2.

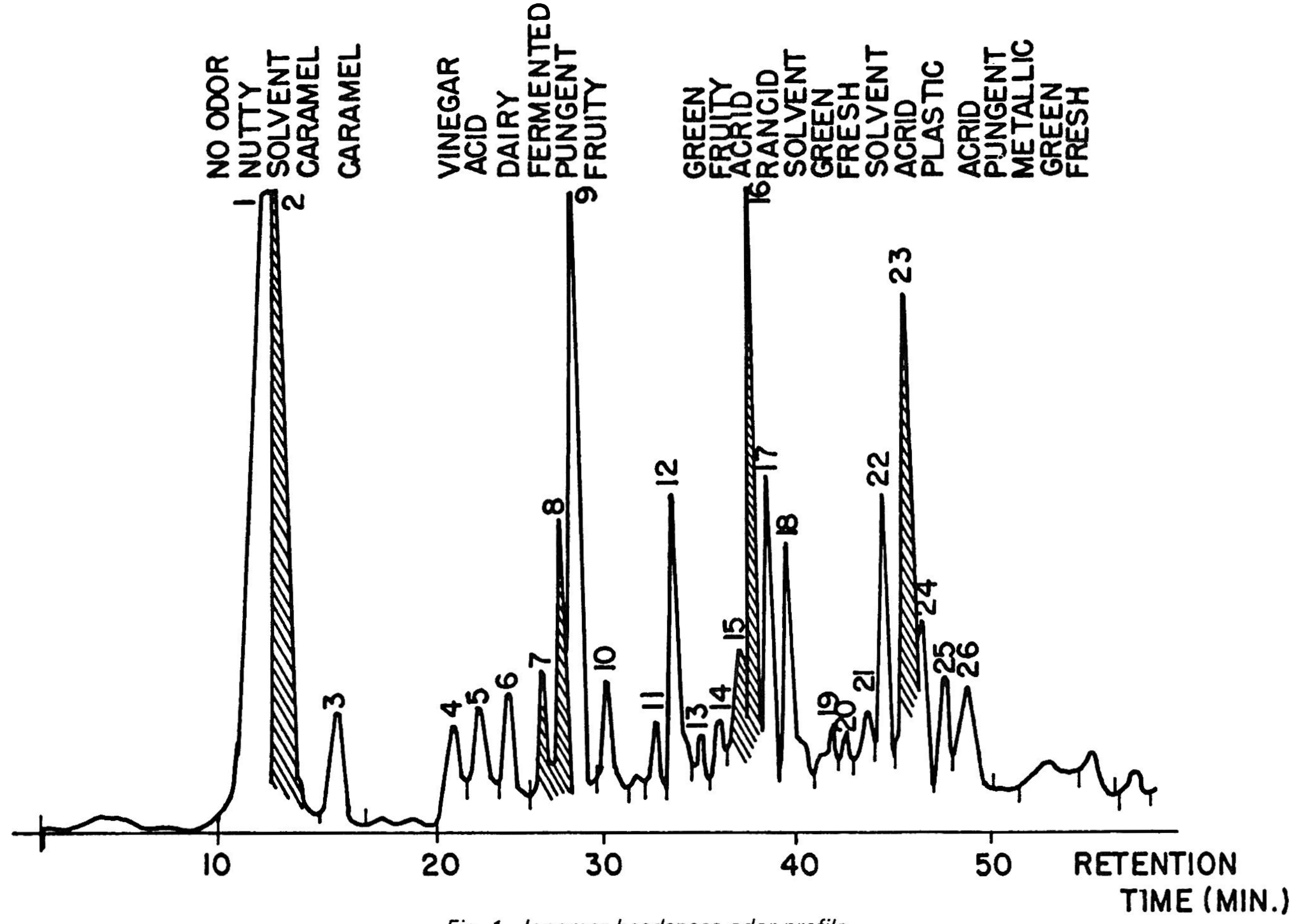

Fig. 1—Ionomer headspace odor profile.

the GC/MS gave better response with the samples prepared at 150°C.

The analysis of the resin using SE 30 column by GC/FID showed very poor resolution, and few compounds were identified from the GC/MS analysis. A more polar column, OV 351, improved the resolution for both GC/FID and GC/MS. Most of the identified compounds were long chain hydrocarbons that were probably formed during the extrusion of the ionomer/foil lamination.

According to Hansen (1970), the thermal decomposition of polyethylene created fragments of widely varying molecular weight up to about 700. This author also stated that except for polyisobutylene, other olefin polymers followed the random scission-intramolecular transfer pattern displayed by high-pressure polyethylene. The morphology of the ethylene ionomer as based on the cluster model (Longworth, 1978) with crystalline and ionic regions connected by a matrix of hydrocarbon could explain the detection of the long chain hydrocarbons. Table 1 shows the compounds tentatively identified from the resin with the OV 351 and SE 30 columns. When the laminate samples were analyzed, however, using these two columns a poor separation was obtained and only a few hydrocarbons were tentatively identified. This finding can be explained by considering that the long chain hydrocarbons present in the resin were probably degraded during the extrusion process at high temperature.

The GC/MS analysis of the resin using the SP 1500 column allowed the identification of 25 compounds such as alcohols, aldehydes, and ketones. Those compounds were detected in very small amounts and are listed in Table 1. GC/FID chromatograms with very good resolution were obtained from the headspace of the ionomer laminates with the SP 1500 column. The compounds tentatively identified are listed in Table 1 which showed that many compounds detected in the laminates had been detected in the resin but in much lower concentration. This led to the assumption that the degradation reaction was initiated during pelletization of the resin and continued during the extrusion process although formation during thermal distillation at 150°C was not ruled out in this work.

By using the splitter in the GC/FID with the SP1500 column, it was possible to produce a flavor profile of the ionomer laminate headspace as shown in Fig. 1. None of the substances eluted from the column possessed the same objectionable odor as obtained from the laminates.

No information concerning the volatile products formed during the thermal oxidation of ethylene ionomers has been published. Many compounds detected in the analyzed samples had been previously identified when polyethylene was degraded (Barabas et al., 1976; Hoff and Jacobsson, 1981; Holmstrom and Sorvik, 1974). This observation and the fact that polyethylene is the major component of the ethylene ionomer reinforced the hypothesis of similarities between the mechanisms of degradation of both thermoplastics.

The most probable paths of formation of different classes of organic products during thermal oxidation of low density polyethylene in the temperature range 284–355°C has been discussed (Holmstrom and Sorvik, 1974). The reactions considered account for the formation of aldehydes, ketones, alcohols, tetrahydrofuran derivatives, and vinyl and vinylene groups within the polymer melt. These reactions adequately explained the results obtained in this ethylene ionomer study.

Correlation of the formation of compounds with the laminate manufacturing process

100°C was the headspace collection temperature used for the correlation studies. This lower temperature was selected to assure that any degradation compounds detected were formed during the manufacturing process of the ionomer and not during the headspace concentration step.

From the GC/FID chromatograms obtained using the SP

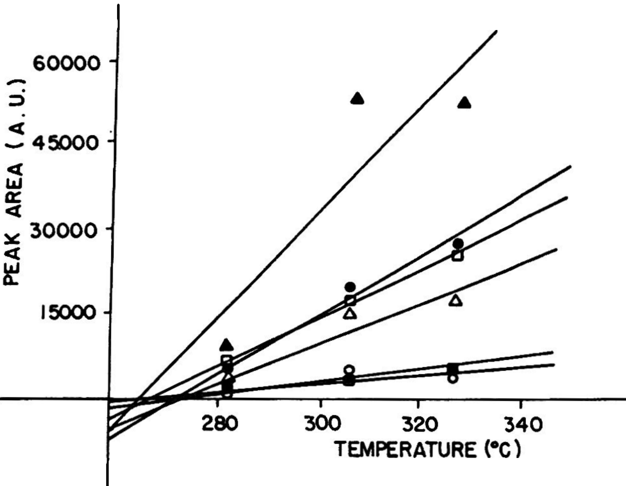

Fig. 2—Peak area vs extrusion process temperature for ionomer samples prepared at 100°C. ▲ Peak #2, 2-butanone; ○ Peak #7, 2-methyl-2-butenal; △ Peak #8, 2-pentanone; ■ Peak #15, 3-hexanone; ● Peak #16, 2-hexanone; □ Peak #23, 3-heptanone.

Table 2—Statistical results of sensory evaluation tests

	Panel Score	
	Test 1[a]	Test 2[b]
Ionomer A	3.3[c]	2.5
Ionomer B	4.8	4.0
Ionomer C	5.5	4.5
Coded Control	1.6	1.6

[a] Judges = 24; F = 3.81; Significant at 95%; LSD = 2.1
[b] Judges = 23; F = 2.51; Significant at 90%; LSD = 2.0
[c] 1 = No difference from Control; 9 = Extreme difference fron Control

1500 column, six peaks were selected to be used in any further calculations and plots. Those peaks were chosen based on the odor profile and tentative identification of the compounds. All had retention times in the range of bad odor components. They are listed in Fig. 2. The peak areas are means from duplicate experiments.

In Fig. 2 the areas of the six selected peaks are plotted versus the extrusion process temperature of the three laminates. The differences in the amount of compounds formed due to the processing conditions used are related to the positive slope of all curves.

The sensory evaluation tests were run in duplicate at 1-wk intervals. In the first test twenty-four judges were able to correctly identify the coded control; in the second test, twenty-three judges identified the control.

The panel scores on each sample were calculated. Data were analyzed using an analysis of variance and the least significant difference between panel scores calculated. The results are tabulated in Table 2.

In each test, all laminate-packaged samples were significantly different from the control. Ionomer laminates A and C were significantly different from each other. There was no significant difference between samples B and C in either test.

Those results confirmed the findings of the GC/FID chromatograms and led to the conclusion that the formation of degradation compounds in the ionomer laminates was dependent on the resin processing conditions.

Instrumental and sensory data correlation

The statistical correlation of instrumental and sensory data involved the area of the six selected peaks of the GC/FID chromatograms from ionomers A, B and C, prepared at 100°C, and the sensory evaluation panel scores. Multivariate regression analysis was applied. R-SQUARE procedure was used. The higher coefficient of correlation obtained was 0.926, with a three variable model. No further improvement was possible because of the high colinearity of the independent variables (peak areas). The correlation obtained could be considered very satisfactory, although only a few samples were analyzed.

The major conclusion of this study was that in the absence of processing aids, extrusion process conditions were responsible for the amount of volatile compounds formed in the ionomer by degradation reactions.

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Inhibition of *Clostridium sporogenes* PA 3679 and Natural Bacterial Flora of Cooked Vacuum-Packaged Bratwurst by Sodium Acid Pyrophosphate and Sodium Tripolyphosphate With or Without Added Sodium Nitrite

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ABSTRACT

Survival and growth of inoculated *Clostridium sporogenes* PA3679 and natural aerobic and anaerobic bacterial flora were studied in refrigerated (5°C) and subsequently temperature abused (24–25°C), cooked, vacuum-packaged bratwurst containing 0.5% sodium acid pyrophosphate (SAPP) or sodium tripolyphosphate (STPP) with or without sodium nitrite. Phosphates alone or combined with nitrite did not affect aerobic bacterial counts but resulted in reduced clostridial and anaerobic counts at 5°C. Upon temperature abuse, inhibition of all bacteria by SAPP was significant ($P<0.05$) for up to 48 hr and greatly enhanced by 100 ppm but not by 50 ppm sodium nitrite, whereas STPP lost its antimicrobial properties after 24 hr. Soluble orthophosphate levels had a positive correlation with bacterial inhibition in SAPP-treated bratwurst.

INTRODUCTION

RECENT WORK on the antimicrobial properties of poly- and pyrophosphates added to cooked, vacuum-packaged bratwurst has demonstrated that 0.5% sodium acid pyrophosphate (SAPP) and, to a lesser extent, sodium tripolyphosphate (STPP) effectively inhibited growth of aerobic and anaerobic bacteria, including inoculated *Clostridium sporogenes* PA3679, when the bratwurst was removed from refrigerated storage at 5°C and placed under abuse-temperature conditions (24–25°C) for up to 48 hr (Molins et al., 1985a). This effect was not observed when tetrasodium pyrophosphate or glassy sodium polyphosphate was used at the same level. Nelson et al. (1983) had previously found that 0.4% SAPP used in combination with 40 ppm sodium nitrite and 0.2% sorbic acid or 0.26% potassium sorbate delayed toxin production by *Clostridium botulinum* in mechanically deboned chicken-meat frankfurter emulsions at 27°C to a greater degree than nitrite-sorbate alone. Wagner and Busta (1983) later confirmed the added antibotulinal activity provided by 0.4% SAPP in beef/pork frankfurter emulsions containing 40 ppm sodium nitrite and 0.26% potassium sorbate. The reported aerobic mesophilic bacterial counts were not affected.

Most studies concerning the antimicrobial properties of poly- and pyrophosphates in meats, however, have overlooked the possible influence of naturally occurring phosphatases on the level of phosphates being tested. Rapid enzymatic hydrolysis of the condensed phosphates to shorter-chain compounds and eventually to orthophosphates has been documented by Awad (1968) and by Sutton (1973). Adaptation of a method developed by Dick and Tabatabai (1977) for the measurement of soluble orthophosphates in the presence of labile organic and inorganic phosphorus compounds enabled us to follow the hydrolysis of SAPP and STPP in meats. Even short delays in meat phosphatase inactivation by cooking temperatures reduced the level of SAPP or STPP considerably (Molins et al., 1985b).

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The objectives of the present study were to determine and compare the effects of SAPP and STPP alone or in combination with high and reduced levels of sodium nitrite on the survival and growth of *C. sporogenes* PA3679 and of the spoilage flora of cooked, vacuum-packaged bratwurst under refrigeration and temperature-abuse conditions, and to relate the effects to residual levels of the condensed phosphates and to those of orthophosphates generated through poly- or pyrophosphate breakdown.

MATERIALS & METHODS

Bratwurst preparation

A 300-lb (136.4 kg) batch of ground pork butts in 12-lb (5.5 kg) portions was obtained from the Iowa State Meat Science Laboratory, packaged in polyethylene freezer bags, twist-tied, frozen in an air-blast freezer to −28°C and kept frozen until needed. Before preparation, 12-lb (5.5 kg) portions were allowed to thaw for 48 hr in a walk-in cooler at 2–4°C. Bratwurst spice mix (Saratoga Specialties, Elmhurst, IL) was added to the meat and blended for 5 min at medium speed by means of a Hobart C-10 mixer. The spice mix used was 2% (w/w), equivalent to 1.7% NaCl in the product. Portions of 500g meat were weighed and each received 26.5 mL 10% (w/v) solution of a single phosphate previously sterilized by filtration through a 22 μ Millipore membrane filter (Millipore Corp., Bedford, MA) so as to obtain the desired concentration of 0.5% in the product. The phosphates used were sodium acid pyrophosphate (SAPP) (Stauffer Chemical Co., Westport, CT) and sodium tripolyphosphate (STPP) (Monsanto, St. Louis, MO). Batches of the mix then received either sodium nitrite as a filter-sterilized 10% (w/v) aqueous solution properly diluted with sterile distilled water to provide a concentration of 50 or 100 ppm in the product, or the equivalent volume of sterile distilled water to equalize the volume of added water in all batches (46.5 mL water/500g meat, equivalent to ca. 1%). Control sausages were prepared with no additives or with each level of nitrite and no phosphate and with each phosphate but no nitrite. After being blended for 1 min at each low and high speeds in a Kitchen Aid Model 4 mixer, the 500-g batches were divided in half; one-half was inoculated and the second half received an amount of sterile distilled water equal to the volume of inoculum added to the first portion. The inoculum consisted of 5 mL of a *Clostridium sporogenes* PA3679 spore suspension in sterile, deionized water prepared according to the procedure outlined by Swank (1971) and targeted to give approximately 10000 spores/g of sausage. The culture was obtained from the ISU Food Technology Dept. collection and selected for rhizoid colony formation to facilitate differentiation from other anaerobic bacteria during plate counting. The mixes were stuffed into 2.5 cm (1-in) diam, edible collagen casings (Devro, Inc., Sommerville, NJ) by means of an Oster Food and Meat Grinder (Oster, Milwaukee, WI). Immediately after being stuffed and tied, each batch of sausage was cooked to an internal temperature of 65.5°C in a separate 3000-mL beaker containing distilled water and kept at 80°C in a Magni Whirl Constant Temperature Bath (Blue M Electric Co, Blue Island, IL) equipped with bottom paddle agitation. The cooking end point was determined by inserting a copper-constantan thermocouple longitudinally through one bratwurst of each batch and monitoring the internal temperature with a Hewlett Packard 3476A Multimeter Potentiometer. Cooking losses that could have affected the level of phosphates and/or nitrite were

previously determined and found to be negligible (<1.5%). After cooking, the bratwurst was wrapped in aluminum foil, rapidly cooled to an internal temperature of 4–5°C, separated and placed in small, individual, polyethylene bags to avoid cross-contamination. A packaged sausage from each treatment, for a total of nine sausages, was placed in a larger Curlon 892 (Curwood Inc., New London, WI) vacuum packaging bag (O_2 permeability <1 mL/645 cm^2/24 hr at 22.8°C and 0% RH) and vacuum sealed in a Multivac MG-2 packaging machine. The bags were stored for 7 days in a display case at about 5°C, followed by 48 hr of temperature abuse (24–25°C). The order of batch preparation was randomly assigned, and all experiments were replicated three times.

Sampling

Packages of sausages were taken from storage on days 0, 1, 3, 5, and 7 and on days 8 and 9 (corresponding to 24 and 48 hr of temperature abuse at 24–25°C) for microbiological examination. Sausage was weighed in 30-g amounts and blended in a sterile plastic bag with 270 mL of 0.1% sterile peptone water for 2 min by means of a Stomacher Lab Blender 400 (Tekmar Company, Cincinnati, OH). Serial dilutions were then made according to standard methods. Trypticase soy agar (TSA, BBL) was used to plate aerobic mesophilic (48 hr, 30°C) and psychrotrophic (10 days, 5°C) bacterial populations. TSA was also used for total anaerobic counts, whereas TSA containing 0.1% soluble starch (Fisher Scientific Co., Fair Lawn, NJ) was the medium used to determine anerobic viable spore counts after heat shocking the appropriate dilutions at 80°C for 20 min (Swank, 1971). The *C. sporogenes* identity of typical rhizoid colonies, enumerated from total anerobic, vegetative cell and spore count plates, based on morphology, was ascertained by randomly taking 10 such colonies from counted plates on a daily basis, examining cells microscopically and culturing in cooked meat medium (BBL; 37°C, 48 hr). Gas formation, proteolysis, blackening of the medium and terminal, single-spore formation were taken to indicate positive identification. No typical rhizoid colonies were found in uninoculated control sausages. BBL Gas Pak Anaerobic Systems were used to obtain anaerobic incubation conditions (48 hr, 37°C). The pH of the sausage was determined throughout the sampling period with a Radiometer 28 meter equipped with an Orion 9163 probe. Soluble orthophosphate levels in the sausage were determined on every sampling day by the method of Dick and Tabatabai (1977) as adapted by Molins et al. (1985b). Sampling order was randomized among treatments.

Plate count data were transformed to logarithms and analyzed by means of an SAS program with GLM option. Comparison of means was based on Duncan's multiple range test.

RESULTS & DISCUSSION

AFTER 7 days of refrigerated storage at 5°C, sausages formulated with SAPP or STPP, alone or combined with 50 or 100 ppm $NaNO_2$, had significantly (P<0.05) lower total anaerobic and PA3679 counts than control sausages containing no additives or only nitrite (Fig. 1, 2, 6 and 7). However, the absolute value of the differences in clostridial and total anaerobic counts between days 0 and 7 of refrigerated storage was small (<1 log cycle) within each individual phosphate treatment. No significant (P>0.05) treatment effect could be detected on aerobic mesophilic or psychrotrophic bacterial counts throughout the 7-day refrigerated storage period (Fig. 4 and 5).

When the sausages were allowed to remain at 24–25°C for 24 hr after refrigeration, however, those containing no additives or only nitrite at either level had significantly (P<0.05) higher numbers of total anaerobes (Fig. 1 and 6), PA3679 vegetative cells (Fig. 2 and 7), aerobic mesophilic (Fig. 4) and psychrotrophic (Fig. 5) bacteria than phosphate-treated bratwurst. At 24 hr, SAPP alone significantly (P<0.05) inhibited growth of anaerobes (Fig. 1 and 2), followed in decreasing order of effectiveness by STPP/50 ppm $NaNO_2$ (Fig. 6 and 7) SAPP/100 ppm $NaNO_2$ (Fig. 1 and 2) and STPP alone (Fig. 6 and 7). The presence of SAPP/100 ppm nitrite and of SAPP alone was also significantly (P<0.05) inhibitory to all aerobic bacteria at 24–25°C (Fig. 4 and 5) and so was, to a lesser extent, that of SAPP/50 ppm nitrite, STPP alone and STPP/50 ppm nitrite (not shown graphically). The SAPP/100 ppm

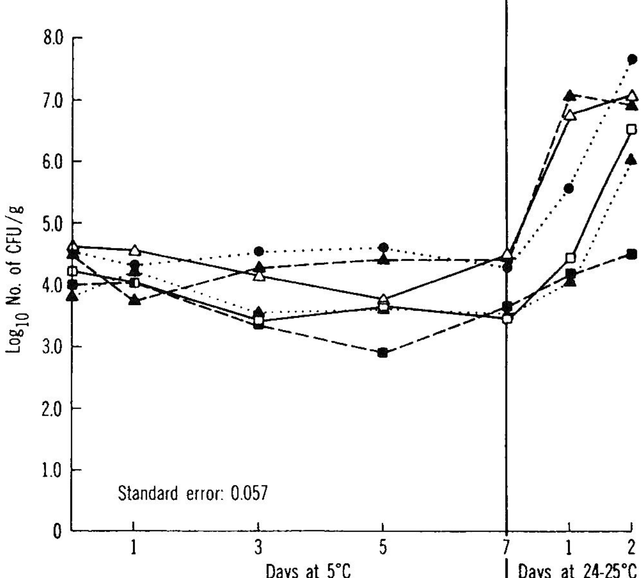

Fig. 1—Total anaerobes in SAPP/NO_2 combinations: ● · · · · · ● Control (No Additives); ▲ · · · · · ▲ (0.5% SAPP + 0 ppm NO_2); △ —— △ (0% SAPP + 50 ppm NO_2); ▲ - - - ▲ (0% SAPP + 100 ppm NO_2); □ —— □ (0.5% SAPP + 50 ppm NO_2); ■ - - - ■ (0.5% SAPP + 100 ppm NO_2).

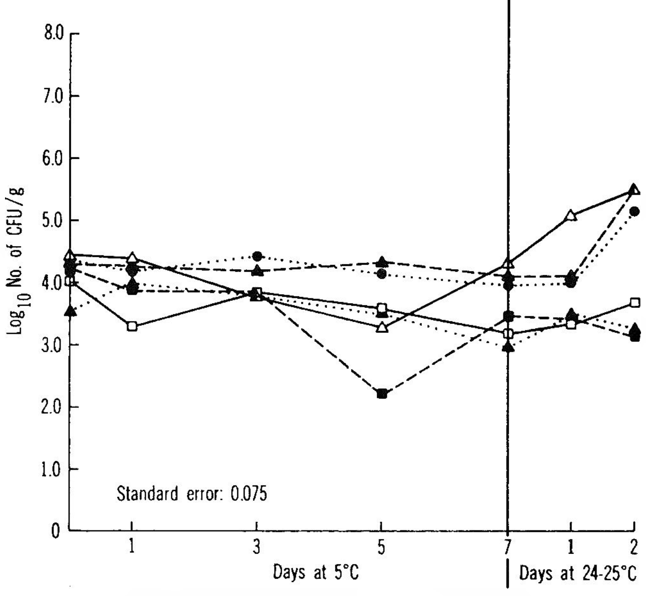

Fig. 2—C. Sporogenes PA3679 counts in SAPP/NO_2 combinations: ● · · · · · ● Control (No Additives); ▲ · · · · · ▲ (0.5% SAPP + 0 ppm NO_2); △ —— △ *(0% SAPP + 50 ppm NO_2); ▲ - - - ▲ (0% SAPP + 100 ppm NO_2); □ —— □ (0.5% SAPP + 50 ppm NO_2); ■ - - - ■ (0.5% SAPP + 100 ppm NO_2).*

nitrite combination also seemed to inhibit PA3679 spore germination for 24 but not for 48 hr at 24–25°C, whereas clostridial spore germination took place shortly after the sausage was placed under abuse-temperature conditions with all other treatments (Fig. 3). A comparison of PA3679 vegetative cell and viable spore counts in SAPP/NO_2 combinations (Fig. 2 and 3, respectively) indicates that 0.5% SAPP, with or without nitrite, allowed spore germination but inhibited clostridial growth at 24–25°C for more than 24 hr. STPP, however, did not inhibit PA3679 growth after 24 hr of temperature-abusing the sausage (Fig. 7). During the first 24 hr of abuse-temperature holding, *C. sporogenes* growth was clearly seen only in sausages containing the lower level of sodium nitrite (50 ppm)

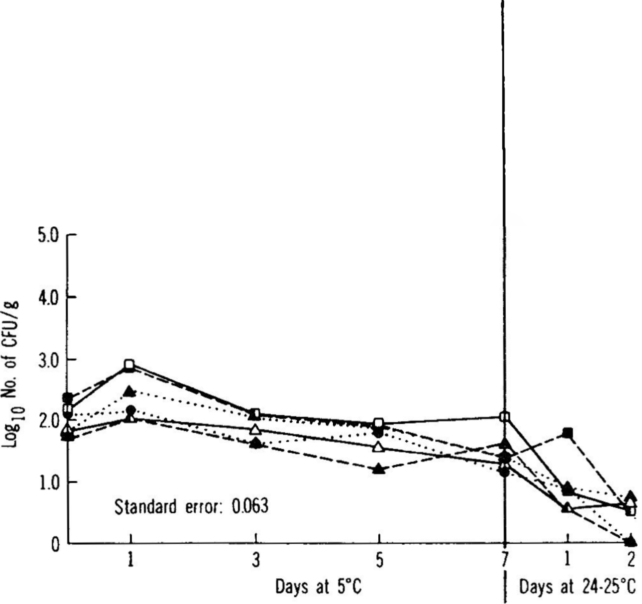

Fig. 3—C. Sporogenes PA3679 viable spores in SAPP/NO_2 combinations: ● · · · · · ● Control (No Additives); ▲ · · · · · ▲ (0.5% SAPP + 0 ppm NO_2); △ —— △ *(0% SAPP + 50 ppm NO_2)*; ▲ – – – ▲ *(0% SAPP + 100 ppm NO_2)*; □ —— □ *(0.5% SAPP + 50 ppm NO_2)*; ■ – – – ■ *(0.5% SAPP + 100 ppm NO_2).*

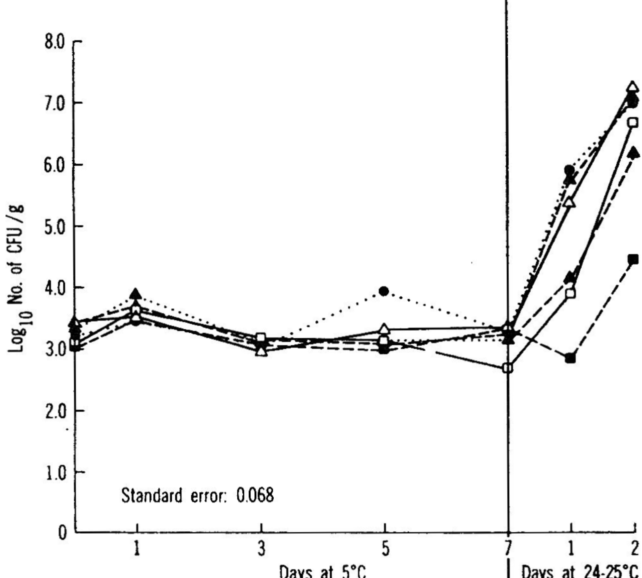

Fig. 4—Total aerobic mesophilic counts in SAPP/NO_2 combinations: ● · · · · · ● *Control (No Additives)*; ▲ · · · · · ▲ *(0.5% SAPP + 0 ppm NO_2)*; △ —— △ *(0% SAPP + 50 ppm NO_2)*; ▲ – – – ▲ *(0% SAPP + 100 ppm NO_2)*; □ —— □ *(0.5% SAPP + 50 ppm NO_2)*; ■ – – – ■ *(0.5% SAPP + 100 ppm NO_2).*

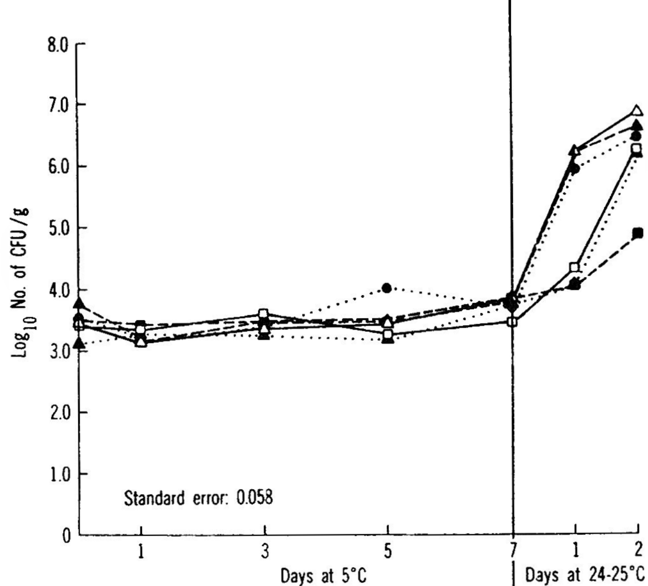

Fig. 5—Psychrotrophic counts in SAPP/NO_2 combinations: ●·····● *Control (No Additives)*; ▲ · · · · · ▲ *(0.5% SAPP + 0 ppm NO_2)*; △ —— △ *(0% SAPP + 50 ppm NO_2)*; ▲ – – – ▲ *(0% SAPP + 100 ppm NO_2)*; □ —— □ *(0.5% SAPP + 50 ppm NO_2)*; ■ – – – ■ *(0.5% SAPP + 100 ppm NO_2).*

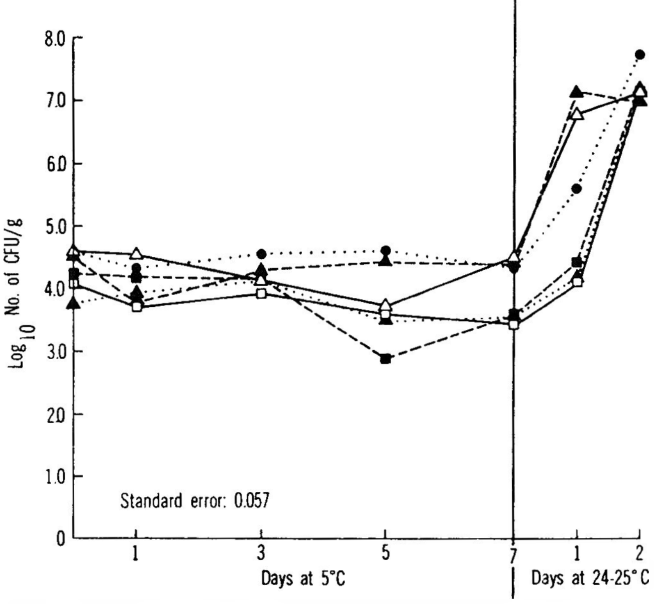

Fig. 6—Total anaerobes in STPP/NO_2 combinations: ● · · · · · ● *Control (No Additives)*; ▲ · · · · · ▲ *(0.5% STPP + 0 ppm NO_2)*; △ —— △ *(0% STPP + 50 ppm NO_2)*; ▲ – – – ▲ *(0% STPP + 100 ppm NO_2)*; □ —— □ *(0.5% STPP + 50 ppm NO_2)*; ■ – – – ■ *(0.5% STPP + 100 ppm NO_2).*

and no phosphate, but after 48 hr of elevated-temperature storage, clostridial growth was detected in all sausages except those treated with SAPP alone or combined with 100 ppm nitrite. At that time, all sausages except those containing SAPP were visibly spoiled, as evidenced by textural breakdown and strong off-odors.

Upon 48 hr of temperature abuse, the combination SAPP/100 ppm $NaNO_2$ had a very strong, significant ($P<0.05$) inhibitory effect on the growth of all types of bacteria under study, particularly on anaerobic and aerobic mesophilic microorganisms (Fig. 1, 2, 4, and 5). No similar effect on aerobic mesophilic counts was reported by Wagner and Busta (1983) in beef/pork frankfurter emulsions containing 40 ppm $NaNO_2$, 0.4% SAPP and 0.26% potassium sorbate. SAPP alone or combined with 50 ppm nitrite also significantly ($P<0.05$) affected anaerobic bacterial growth in the bratwurst but to a smaller extent than in combination with the higher level of $NaNO_2$. The magnitude of the anitmicrobial activity of the SAPP/100 ppm nitrite treatment after 48 hr of temperature abuse of the bratwurst was noteworthy. Bacterial counts in sausages with that combination were 2–3 log cycles below those found in control samples and only 1–1.5 log cycles higher than bacterial numbers in the bratwurst at the end of the refrigerated storage period at 5°C. In contrast, treatments that

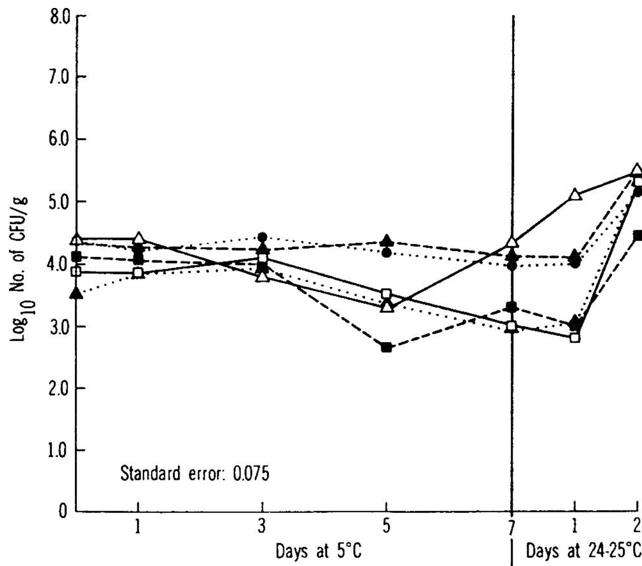

Fig. 7—C. Sporogenes PA3679 counts in STPP/NO_2 combinations: ● · · · · · ● Control (No Additives); ▲ · · · · · ▲ (0.5% STPP + 0 ppm NO_2); △ —— △ *(0% STPP + 50 ppm NO_2); ▲ – – – ▲ (0% STPP + 100 ppm NO_2); □ —— □ (0.5% STPP + 50 ppm NO_2); ■ – – – ■ (0.5% STPP + 100 ppm NO_2).*

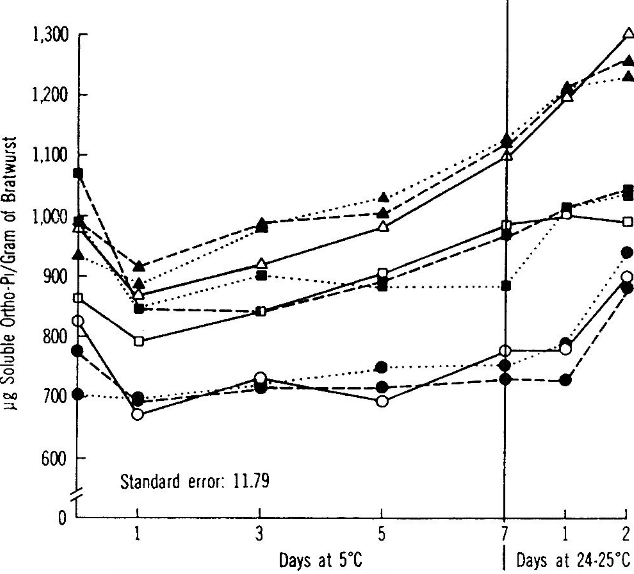

Fig. 8—Soluble orthophosphate content of bratwurst: ● · · · · · ● Control (No Additives); ○ · · · · · ○ (No Phosphate + 50 ppm NO_2); ● – – – ● (No Phosphate + 100 ppm NO_2); ■ · · · · ■ (0.5% STPP + 0 ppm NO_2); □ —— □ (0.5% STPP + 50 ppm NO_2); ■– – –■ (0.5% STPP + 100 ppm NO_2); ▲ · · ·; · ▲ (0.5% SAPP + 0 ppm NO_2); △ —— △ (0.5% SAPP + 50 ppm NO_2); ▲ – – – ▲ (0.5% SAPP + 100 ppm NO_2).

included STPP ceased to have any antibacterial effect when bratwurst was exposed to 24–25°C for more than 24 hr. The effects of STPP treatments on viable PA3679 spore counts and on aerobic mesophilic and psychrotrophic bacterial numbers did not differ significantly (P>0.05) from those corresponding to SAPP treatments until after 24 hr of temperature abuse (data not presented graphically).

Soluble orthophosphate levels present in the sausage throughout the 5°C holding period and upon temperature abuse are shown in Fig. 8 for all treatments. A decrease in the level of soluble orthophosphates took place in all samples except controls with no additives within 24 hr of manufacture and storage at 5°C, possibly as a result of chelation, complexing with proteins or other reactions with meat components (Sherman, 1961; Nikkila et al., 1967; Tenhet et al., 1981). The initial disappearance of soluble orthophosphates was followed by a gradual increase over time, similar to that observed in beef by Awad (1968), until the original level was reestablished and surpassed within 3–5 days in SAPP-containing bratwurst. Slower increases were observed in treatments that included STPP, whereas the initial loss of soluble orthophosphates in sausages with only nitrite was not recovered until more than 24 hr of temperature abuse had elapsed. Sausages containing SAPP had significantly (P<0.05) higher levels of soluble orthophosphates since manufacture and as a group than did STPP-treated bratwurst. The latter, in turn, had significantly (P<0.05) higher levels than controls with no additives or only nitrite. A grouping of treatments according to type of phosphate added can be observed throughout the 7-day refrigerated storage at 5°C and beyond in Fig. 8.

A negative correlation was found between soluble orthophosphate content and the mean of all bacterial counts from day 1 through day 8 (24 hr at 24–25°C) in SAPP-treated bratwurst. Correlation coefficients were −0.87, −0.92, −0.68, and −0.78 for SAPP alone, SAPP/50 ppm $NaNO_2$, SAPP/100 ppm $NaNO_2$ and all SAPP-treated sausages as a group, respectively, suggesting a possible participation by orthophosphates in the overall antibacterial effects exhibited by SAPP. Similar correlations were not obtained with STPP. In the case of SAPP treatments, if the the total soluble orthophosphate increase measured at the end of the experimental period was attributed to SAPP breakdown, approximately 0.37% SAPP (w/w) would have remained in the bratwurst (Molins et al., 1985b).

Although phosphate-induced pH differences in bratwurst cannot be discounted as a possible factor in the antimicrobial effects of the various phosphate treatments studied, such differences appeared to be too small to account for the observed inhibitory activity of phosphates. Bratwurst pH values showed little variation (<0.6 pH unit) throughout the experimental period. Overall mean pH values were 6.31, 5.98, and 6.51 for control sausages (no additives or only nitrite), SAPP- and STPP-treated sausages, respectively.

Whether orthophosphates enhance the antibacterial properties of SAPP or simply prevent possible enzymatic, chemical and/or bacterial depletion of residual SAPP to noninhibitory levels remains to be elucidated and appears worthy of future study. Large increases in soluble orthophosphate content in sausages to which SAPP has been added might also be only indirectly related to bacterial inhibition in meat systems. Unlike other phosphates, SAPP induces a decrease in pH values of meat and meat products (Terrell et al., 1982; Knipe, 1982; Hargett et al., 1980; Ivey and Robach, 1978), and its overall mode of action against bacteria may consequently be different from that of the alkaline phosphates. From this study and previous experiments with uncooked bratwurst, however, it was suggested that cooking SAPP- and STPP-containing bratwurst immediately after preparation was essential in retaining the antimicrobial properties of these phosphates, and that their use might provide a safety margin against clostridial growth in cooked meat products.

Further study of the possible bactericidal properties of SAPP and STPP on clostridia and other anaerobic bacteria in cooked, vacuum-packaged bratwurst under prolonged refrigerated storage conditions seems also justified.

CONCLUSIONS

ADDITION of 0.5% sodium acid pyrophosphate (SAPP) or sodium tripolyphosphate (STPP) did not significantly (P>0.05) improve the microbiological quality of cooked, vacuum-packaged bratwurst held at 5°C for 7 days, as measured by unaffected psychotrophic bacterial counts, but significantly (P<0.05)

reduced the number of surviving anaerobic bacteria (including inoculated *C. sporogenes* PA3679).

A combination of 0.5% STPP/50 ppm $NaNO_2$ effectively inhibited anaerobic and clostridial growth in bratwurst exposed to an abuse temperature of 24–25°C for 24 hr but not for 48 hr.

The combination 0.5% SAPP/100 ppm $NaNO_2$ was significantly ($P<0.05$) inhibitory to inoculated *C. sporogenes* PA3679 and to the natural anaerobic and aerobic bacterial flora of bratwurst subjected to 24 and 48 hr of temperature abuse (24–25°C). Although less effective in this study, the use of combinations of SAPP and lower levels of sodium nitrite as antimicrobials in cooked meat products appeared worthy of future research.

Increased levels of soluble orthophosphates had a positive correlation with bacterial growth inhibition in SAPP-containing bratwurst, suggesting that orthophosphate formation from SAPP hydrolysis might be involved in the overall antibacterial effect of SAPP.

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six times compared with cellulase alone. Lowering the pH of the tissue with HCl drastically reduced the dissociation of the functional carboxyl groups in the polyuronide and increased hydrogen bonds interactions in the polymer Doesberg (1965). This effect is supposed to alter steric changes in the structure of the pectin in the cell wall, thereby enabling cellulase to penetrate partially through this barrier. In order to eliminate the effect of pectic substances in the cell wall, we gently extracted the pectic substances in the cell wall at 25°C with 0.05 NaOH solution which included 0.2% EDTA (Table 1). Treating the tissue with NaOH and EDTA or with cellulase did not release much glucose from the tissue. Extracting the pectic substances from the tissue with NaOH and EDTA and then treating the tissue with cellulase, released the greatest amount of glucose from the cell wall into the aqueous extract. The amount of glucose which was released by NaOH, EDTA and cellulase was found to be 10 times more than that released by cellulase alone, and 1.7 times more than by the combination of pectinase and cellulase. This further indicates that the pectic substances in the cell wall sterically hinder cellulose hydrolysis, and provides evidence for the assumption that the pectic substances in the cell wall surrounding the hemicellulose and cellulose.

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Characterization and Identification of Raw Beef, Pork, Chicken and Turkey Meats by Electrophoretic Patterns of Their Sarcoplasmic Proteins

H. KIM and L.A. SHELEF

ABSTRACT

Electrophoretic patterns of fresh beef, pork, chicken, and turkey sarcoplasmic proteins were studied using thin layer agarose gel electrophoresis. Creatine kinase isozyme MM and myoglobin bands were used to identify the species. Binary mixtures (5/95, 25/75, 50/50, 75/25, 95/5, % by weight of each species) were also examined by their electrophoretic and densitometric patterns. Electrophoretic patterns remained stable at elevated pH (7.5), but were affected by low pH (4.7). The relative ratios of the bands which characterized each species changed in proportion to the species content in each binary mixture. These ratios make it possible to predict the approximate fraction of each species present in such mixtures.

INTRODUCTION

IMMUNOCHEMICAL (Hayden, 1978, 1979; Uhlenhuth, 1901), chromatographic (Cook and Sturgeon, 1966; Medina and Phillips, 1982) and electrophoretic (Höyem and Thorson, 1970; Tinbergen and Olsman, 1976; Kaiser et al., 1980; Prasad and Misra, 1981) methods have been employed for meat species characterization. The immunological method, first demonstrated by Uhlenhuth in 1901 and practiced with various modifications, requires use of species specific antisera and efficient extraction of antigens. Cook and Sturgeon (1966) reported chemical identification of horse, pork and beef fat by first separating the unsaponifiable matter of meat fat samples and fractionating by column chromatography, and then examining by gas chromatography. Medina and Phillips (1982) attempted to identify beef, pork, chicken, and soy by their peptide patterns after tryptic digestion using thin-layer chromatography and high-performance liquid chromatography. Electrophoretic techniques have been most frequently employed for fish and meat species identification, either by general protein or enzyme patterns. They are officially recommended for the identification of fish species (AOAC, 1984). Either the starch gel zone method, acrylamide disk method or cellulose acetate strip method are suggested, and in each case identification of species is made by comparing patterns to those of authentic samples run simultaneously. Thomson (1961) reported variations in protein patterns between beef and pork, and different cuts of single species gave identical band configurations. Scopes (1968) investigated general protein, myoglobin and glycolytic enzyme patterns of beef, pork and chicken by starch gel electrophoresis. Höyem and Thorson (1970) studied myoglobin band patterns of beef, whale and horse meat by acrylamide gel electrophoresis and characterized the bands by their red color without further staining. Sinclair and Slattery (1982) compared unstained myoglobin bands and sarcoplasmic proteins of beef and pork with isoelectric focusing electrophoresis. Heinert and Klinger (1980) and Prasad and Misra (1981) used esterase patterns for species identification. The former investigated chicken and beef esterase patterns and found some variations within species age and sex groups. More recently,

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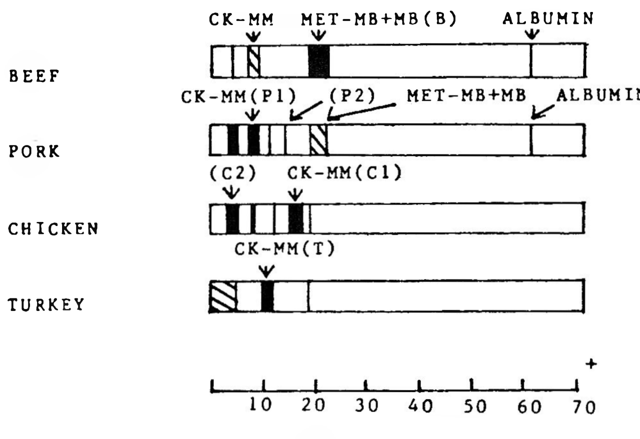


Fig. 1—Schematic presentation of beef, pork, chicken, and turkey sarcoplasmic protein patterns. CK-MM, major creatine kinase isozyme; MetMb, metmyoglobin; MB, myoglobin; B, beef; P_1, major pork CK-MM bands; P_2, unidentified pork band; C_1, major chicken CK bands; C_2, unidentified chicken band, T, turkey.

King and Kurth (1982) stained isoelectric focusing gels for phosphoglucomutase to detect pig, horse, or buffalo meat in beef, while Kaiser et al. (1980) attempted a semiquantitative species determination in binary meat mixtures such as beef and pork by comparing changes in peak dimensions after isoelectric focusing electrophoresis.

The majority of studies to date have been conducted with beef and pork. Although the importance of chicken and turkey as meat sources in mixed meat products has been growing steadily, very few attempts have been made to quantitate them in meat mixtures. The numerous bands which appear in general protein patterns in isoelectric focusing tend to limit the ease of detection, and enzymatic activities of meat samples during the electrophoretic procedures for the quantitative measurements are not easily controlled.

This study was undertaken in order to (1) compare sarcoplasmic protein patterns of beef, pork, chicken and turkey by thin layer agarose gel electrophoresis and identify the major proteins, (2) study the effect of pH on the protein patterns and (3) attempt a qualitative and quantitative detection of meat species in binary mixtures.

MATERIALS & METHODS

Meat extracts

Fresh beef, pork, chicken, and turkey meat samples were obtained from meat markets in the Detroit area, minced separately, and 100g meat from a single species, or from a mixture of binary species (5/95, 25/75, 50/50, 75/25 and 95/5% by weight) were prepared. Samples were blended with 30 ml distilled water for 15 sec and centrifuged at 1,500 × *g* for 20 min. Meat extracts for myoglobin testing were prepared with 15 mL distilled water. Supernatants were collected and stored at −20°C. The thawed samples were centrifuged again at 5,000 × *g* for 10 min prior to testing. The pH of the meat extracts was

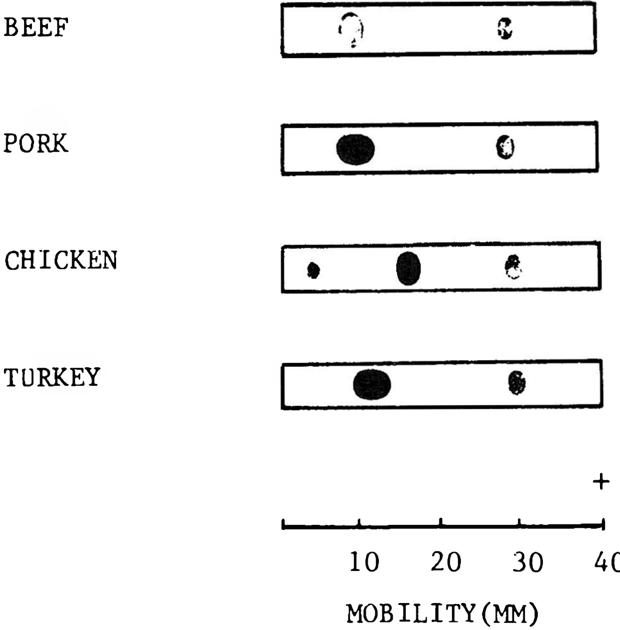


Fig. 2—Schematic presentation of creatine kinase isozyme bands of beef, pork, chicken, and turkey as viewed under UV light. The major band of each species is MM isozyme; the faster moving band is MB.

adjusted with 1N HCl or 1N NaOH added to the meat extract prior to the second centrifugation.

Electrophoretic procedure

Aliquots of meat supernatants for testing myoglobin (3 μl), general protein (2 μL), creatine kinase (1 μL of a 1:15 dilution), and alkaline phosphatase (1 μL of a 1:5 dilution) were applied to an agarose gel with a quantitative microliter sample dispenser. The thin layer agarose gel (Corning Universal Electrophoresis Film, Palo Alto, CA) for general protein and myoglobin contained (w/v): 1% agarose, 5% sucrose, and 0.035% EDTA disodium salt in 0.065 M barbital, pH 8.6. Electrophoresis was carried out with 0.05M barbital buffer, 0.035% EDTA, pH 8.35, for 100 min, using a Corning Cassette Electrophoresis Cell and a fixed 90 V power supply. For general proteins, the gel was stained in 0.2% Amido Black 10B in 5% acetic acid for 15 min, dried at 65°C, cleared in 5% acetic acid and dried again at 65°C. For myoglobin, the bands were stained by the method of Owen et al. (1958) using O-tolidine reagent (Baker Chemical Co., Phillipsburg, NJ). Both general protein and myoglobin bands were scanned by soft laser scanning densitometer (Model SL-504, Biomed Instruments, Inc., Chicago, IL). Peaks on each tracing were divided into 4 areas by their mobilities and quantitated (Anonymous, 1975).

The gel for the enzymes contained (w/v): 1% agarose, and 5% sucrose in 0.065M barbital, pH 8.6. Electrophoresis was carried out with 0.05M barbital buffer, pH 8.35, for 100 min. After electrophoresis, 180 mg creatine kinase substrate (Corning) in 1 mL 0.05M morpholinoethane sulfonic acid (MES) buffer, pH 6.2, with sucrose, or 5 mg alkaline phosphatase substrate: naphthol AS-MX phosphoric acid disodium salt (Corning) in 1 mL 2-amino-2-methyl-1-propanol (AMP) buffer with $MgCl_2$ and sucrose, were applied. The gel was incubated as 37°C for 20 min, and dried at 65°C for 20 min. The fluorescent bands were examined under a UV lamp.

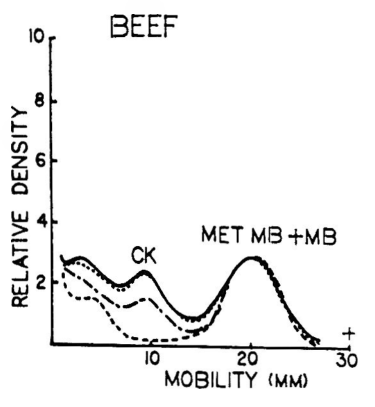
Electrophoresis of myoglobin, creatine kinase, and alkaline phosphatase was carried out simultaneously with electrophoresis of general protein patterns in order to identify the bands. Ten milligram samples of bovine albumin and of bovine creatine kinase (Sigma, St. Louis, MO) were also applied for further identification of bands.

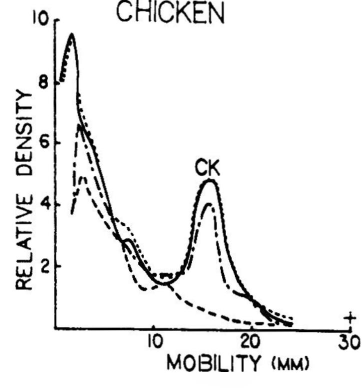
General protein patterns were repeated with five different samples of each meat species. Quantitative identification of species in binary mixtures was repeated twice.

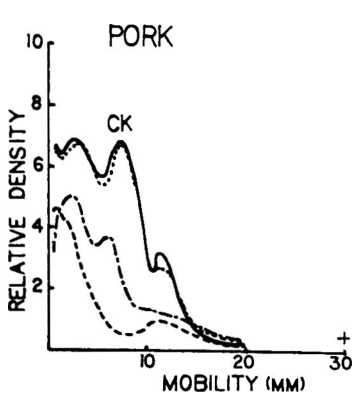
RESULTS & DISCUSSION

Sarcoplasmic protein patterns of beef, pork, chicken and turkey

General protein patterns. Schematic electrophoretic patterns of beef, pork, chicken, and turkey sarcoplasmic proteins







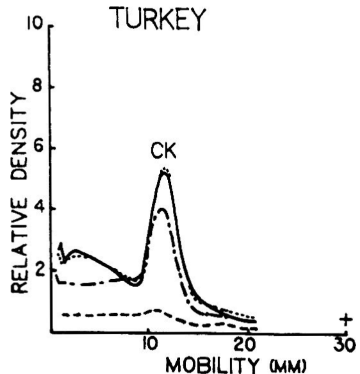


Fig. 3—Effect of pH on densitometric tracings of fresh meat sarcoplasmic proteins. CK — creatine kinase; MetMb + MB, metmyoglobin and myoglobin. Extract pH: · · · ·, 7.5; ——, fresh (5.6-5.9); — · — · —, 5.0; - - - - -, 4.7.

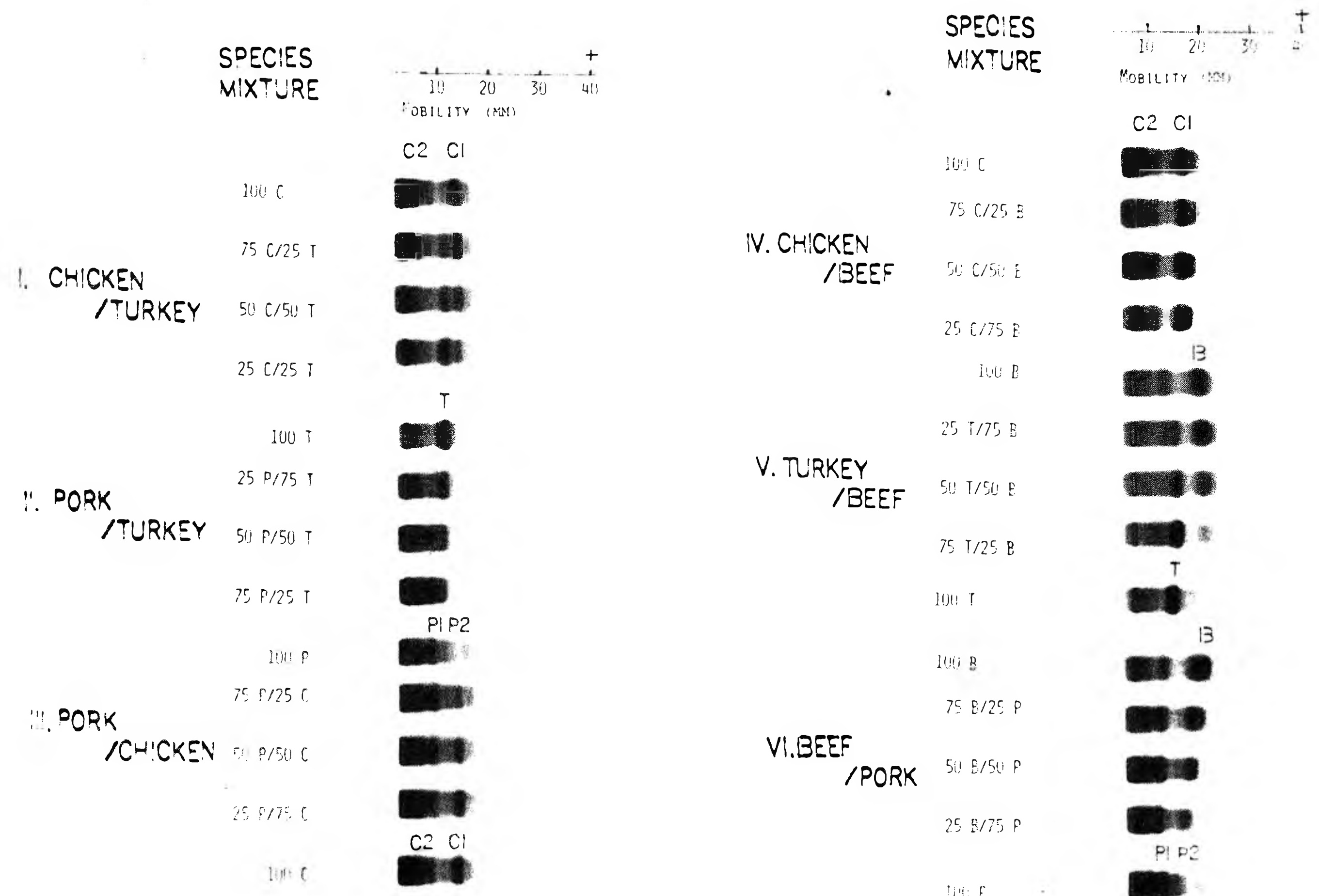


Fig. 4—Electrophoretic patterns of four meat species and their binary mixtures. Bands are: B, beef MetMb + Mb; P_1, pork CK-MM; P_2, pork unidentified; T, turkey CK-MM; C_1, chicken CK-MM; C_2, chicken unidentified.

are shown in Fig. 1. Each meat species had a distinct protein pattern with characteristic concentration and mobilities of proteins. Bands of myoglobin, creatine kinase and alkaline phosphatase were further characterized by specific staining procedures.

Myoglobin patterns. Pronounced myoglobin (Mb) and metmyoglobin (MetMb) bands which migrated 20-21 mm to the anode characterized beef on both general protein (Fig. 1) and Mb stain patterns (data not shown). Turkey (red meat) showed two bands of Mb which migrated 1 mm and 21 mm, while pork and chicken showed only trace amounts of Mb which were influenced by the pigment concentration in the muscle tested. The integrator tracing counts after staining for Mb were 290 for beef and 2 for the main band (1 mm migration) of turkey. Staining of Mb and MetMb bands on agarose gel with O-tolidine reagent were stable for months, unlike the staining of a starch gel which was reported to fade within hours (Owen et al., 1958).

Creatine kinase pattern. The major creatine kinase isozyme (CK-MM) showed a clearly defined, pronounced band by general protein staining in all meat species. These observations for beef, pork, and chicken are in agreement with those published by Scopes (1968). Mobilities of CK-MM were 8, 8, 11, and 16 mm for beef, pork, turkey, and chicken, respectively. The calculated percent of CK-MM bands in total anodic proteins (Anonymous, 1975) was 11.7, 23.4, 25.9, and 40.4 for beef, pork, chicken, and turkey, respectively. Takasawa and Shiokawa (1981) tested pork by DEAE and CM cellulose column chromatography and estimated that the level of the enzyme, based on its specific activity, was 13% of the total sarcoplasmic proteins. According to Watts (1973), CK may represent 10-20% (w/v) of the muscle cytoplasmic proteins. Our data indicate that percent CK in total protein was considerably higher for turkey than for the other species (40.4%). The value for pork (23.4%) is higher than that reported by Takasawa and Shiokawa (1981), who employed different procedures.

When CK isozyme was examined under UV light it showed three isozyme bands for chicken and two bands for each of the other meat species. A schematic presentation of the bands is shown in Fig. 2. CK content in beef was the lowest among the species, as predicted by its observed low CK content on general protein patterns (Fig. 1). The slow moving CK isozyme between the starting point and the major CK band, MM, detected in chicken, is apparently a Macro CK, observed also in human sera (Bohner et al., 1982).

Alkaline phosphatase pattern. Chicken was the only species which showed an alkaline phosphatase band when gels were examined under UV light. However, densitometric tracings revealed very weak peaks for beef, pork, and turkey (data not shown). All of the peaks on the densitometric tracings were at approximately the same location, indicating similar migration rates. Muscle has very low levels of alkaline phosphatase isozymes, as liver, bone, placenta, kidney, and the intestinal mucosa are sources of the enzymes. Consequently, this enzyme cannot be used to characterize muscles from different species, although it may be useful for the detection of organ meats in muscle mixtures. Linear relationship was reported between the amount of serum which contained alkaline phosphatase and the fluorescence produced (Johnson, 1969).

Cathodic bands. When the agarose gel was stained by Fat Red 7B for lipoproteins, each of the meat species showed a

Table 1—Quantitative characterization of four meat species and their binary mixtures[a]

Chicken/Turkey

Chicken/Turkey (%, W/W)		Area under the densitometric tracing curve (%) — Distance from origin (mm) 0 - 6	6 - 9	9 - 14 (T)[b]	14 - 30 (C_1)[c]	T/C
100	0	50.2	11.7	12.3	25.9	0.47
75	25	46.2	10.3	15.8	27.9	0.57
50	50	39.9	8.1	22.1	30.0	0.73
25	75	33.1	11.8	32.1	23.1	1.39
0	100	31.1	12.0	40.4	16.6	2.43

Pork/Turkey

Pork/Turkey (%, W/W)		Area under the densitometric tracing curve (%) — Distance from origin (mm) 0 - 6	6 - 9 (P_1)[d]	9 - 14 (T)	14 - 30	P_1/T
0	100	31.1	12.0	40.4	16.6	0.30
25	75	39.0	17.1	30.2	13.6	0.57
50	50	45.8	21.5	20.2	12.5	1.06
75	25	50.4	21.0	17.3	11.2	1.21
100	0	47.2	23.4	17.4	12.0	1.34

Pork/Chicken

Pork/Chicken (%, W/W)		Area under the densitometric tracing curve (%) — Distance from origin (mm) 0 - 6	6 - 9 (P_1)	9 - 14	14 - 30 (C_1)	P_1/C_1
100	0	47.2	23.4	17.4	12.0	1.95
75	25	47.1	21.4	16.3	15.2	1.31
50	50	50.6	16.8	14.4	18.3	0.92
25	75	50.5	15.4	12.4	21.7	0.71
0	100	50.2	11.7	12.3	25.9	0.45

Chicken/Beef

Chicken/Beef (%, W/W)		Area under the densitometric tracing curve (%) — Distance from origin (mm) 0 - 6 (C_2)[e]	6 - 9	9 - 14	14 - 30 (C_1 + B)[f]	C_2/C_1 + B
100	0	50.2	11.7	12.3	25.9	1.94
75	25	44.8	13.7	10.8	30.7	1.46
50	50	41.8	11.8	10.8	35.6	1.17
25	75	33.3	13.1	11.4	42.1	0.79
0	100	28.5	11.7	14.5	45.2	0.63

Turkey/Beef

Turkey/Beef (%, W/W)		Area under the densitometric tracing curve (%) — Distance from origin (mm) 0 - 6	6 - 9	9 - 14 (T)	14 - 30 (B)[f]	B/T
0	100	28.5	11.7	14.5	45.2	3.12
25	75	25.1	11.0	19.7	44.3	2.25
50	50	24.0	14.0	26.5	35.5	1.34
75	25	25.8	12.7	34.0	27.5	0.81
100	0	31.1	12.0	40.4	16.6	0.41

Beef/Pork

Beef/Pork (%, W/W)		Area under the densitometric tracing curve (%) — Distance from origin (mm) 0 - 6	6 - 9 (P_1)	9 - 14	14 - 30 (B)	B/P_1
100	0	28.5	11.7	14.5	45.2	3.86
75	25	40.1	15.8	12.1	32.0	2.03
50	50	43.6	17.9	15.0	23.6	1.32
25	75	45.1	18.8	13.6	22.6	1.21
0	100	47.2	23.4	17.4	12.0	0.51

[a] Each value is a mean of two or three experiments.
[b] Turkey creatine kinase isozyme MM (CK-MM).
[c] Chicken creatine kinase isozyme MM (CK-MM).
[d] Pork creatine kinase isozyme MM (CK-MM).
[e] Slow moving chicken band (unidentified).
[f] Beef MetMb + Mb.

distinct cathodic band which migrated approximately 10 mm from the origin (data not shown).

Effects of pH on protein patterns. Changes in protein patterns of beef, pork, chicken and turkey were produced by the pH of their extracts and are illustrated in their densitometric tracings (Fig. 3). In each experiment, the pattern of the fresh meats, which ranged in pH from 5.6 (pork) to 5.9 (turkey), was compared with those of extracts at a higher pH value (7.5) and at two lower pH values (4.7 and 5.0). Protein patterns at pH 7.5 remained essentially the same as those of fresh meats. Since this pH typifies putrid meat, electrophoretic patterns of meat from these four animal species revealed little change from freshness to spoilage. On the other hand, when the pH of the meat extracts was lowered, the protein patterns were affected. In general, all the peaks were lowered at pH 5, and CK peaks disappeared completely at a further decrease to pH 4.7. The same results were obtained when the meat pH was reduced to a value of 4.7-5.0 prior to preparation of the extract, and this effect was irreversible.

Sarcoplasmic protein patterns of binary meat mixtures

Electrophoretic patterns of sarcoplasmic proteins from single meat species and from binary mixtures (25/75, 50/50, and 75/25, % by weight), along with their respective densitometric tracings are shown in Fig. 4 and 5. In five of the binary mixtures a single well-defined band or peak, which best characterized the species and its quantity in the mixture, was selected in order to identify it. Thus, major bands or peaks of CK-MM in turkey (T), chicken (C_1), and pork (P_1) and the combined band or peak of MetMb + Mb for beef (B) were selected. The five binary mixtures included those of chicken/turkey (C_1 and T, Fig. 4,I and 5,I), pork/turkey (P_1 and T, Fig. 4,II and 5,II), pork/chicken (P_1 and C_1, Fig. 4,III and 5,III), turkey/beef (T and B, Fig. 4,V and 5,V) and beef/pork (P_1 and B, Fig. 4,VI and 5,VI). Although band or peak C_1 in the chicken/beef mixtures (Fig. 4,IV and 5,IV) changed rapidly with increase in the chicken content, it was too close to band or peak B and the two tended to merge. Band or peak C_2, the slow moving band (migration rate, 3-4 mm) was therefore utilized to better characterize chicken in this mixture. Band C_2 is also well defined in Fig. 4,I and 5,I (chicken/turkey), but less so in Fig. 4,III and 5,III (pork/chicken). With the exception of B (MetMb + Mb) and the secondary band, C_2, all the bands used to identify the species were those of CK-MM (T, C_1, and P_1).

In turkey and beef mixtures (Fig. 4,V and 5,V), the intensity of bands or peaks T and B corresponded to their level in the mixtures. In mixtures of beef and pork (Fig. 4 and 5,VI), two bands or peaks characterized pork (P_1 and P_2). Densitometric tracings of P_1 corresponded clearly to the pork level in the mixtures.

Electrophoretic patterns of binary mixtures containing levels of 5% by weight of the species were also examined. Only 5% chicken in turkey or in pork, and 5% turkey in chicken could be easily identified in the respective electrophoregrams and in the densitometric patterns, because of the distinct differences in mobilities and intensities of CK-MM of chicken, turkey, and pork (8, 11 and 16 mm, and 23.4, 25.9 and 40.4% of total sarcoplasmic proteins), and the absence of interfering bands.

Quantitative identification of species in binary mixtures. For further species characterization by electrophoretic patterns, densitometric data were evaluated by computing the integrator tracings under the densitometric tracing peaks of the meat species and their mixtures. Each tracing was divided into four areas, 0-6, 6-9, 9-14 and 14-30 mm from the start, and the mean values of the integrator tracings were calculated. Percentages of each peak are summarized in Table 1. The unique peaks which characterized the examined species (T, C_1, C_2, P_1, and B) are indicated in the table. Percentage of each peak decreased or increased progressively depending on the species level in the mixture, with two exceptions (C_1 in chicken/turkey and P_1 in pork/turkey). These irregularities were caused by other coinciding peaks which also increased or decreased at the same time, and thus affected the measurements.

In general, in all the binary mixtures, the densitometric trac-

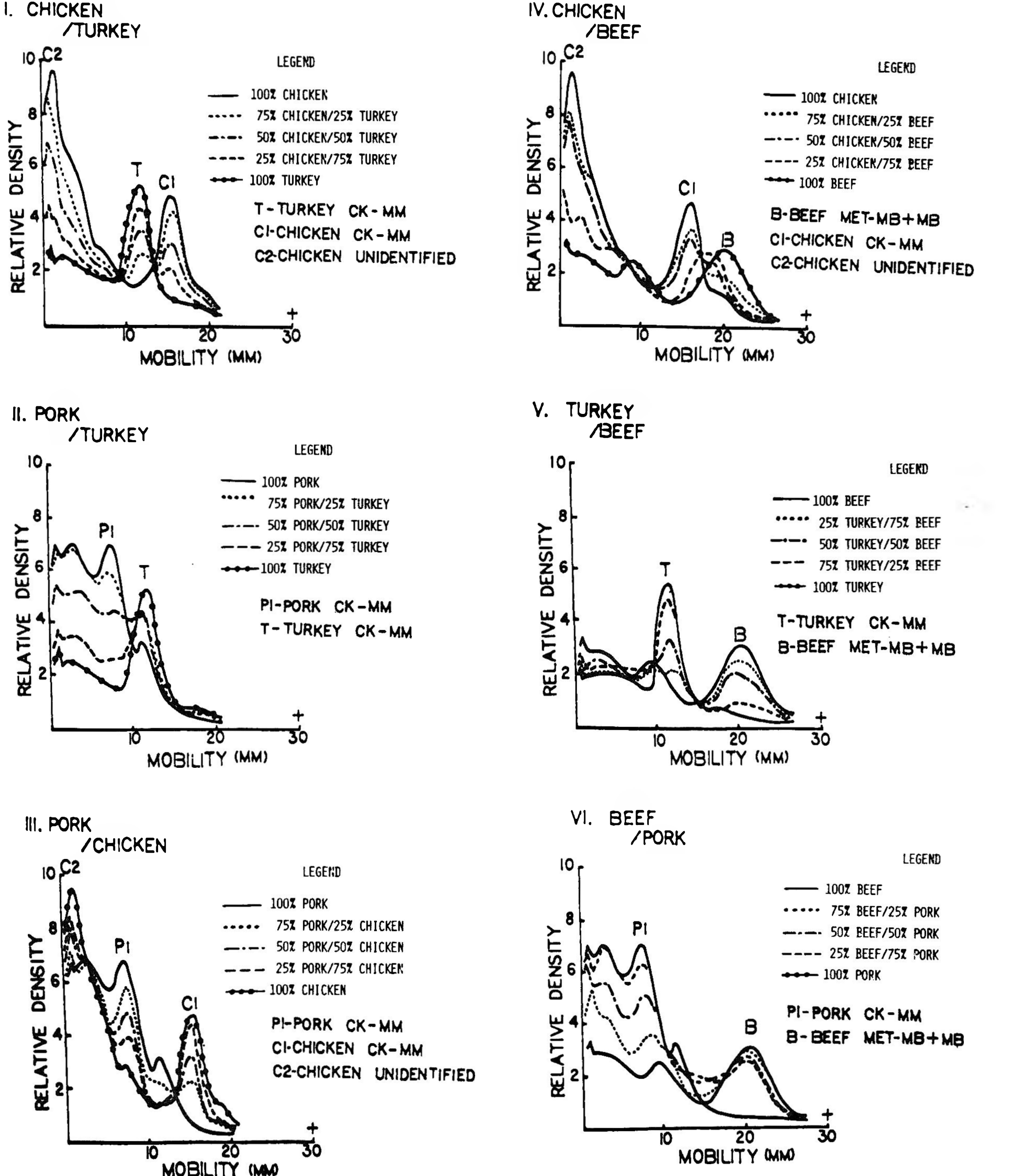


Fig. 5—Densitometric tracings of four meat species and their binary mixtures. Bands are: B, beef MetMb + Mb; P_1, pork CK-MM; P_2, pork unidentified; T, turkey CK-MM; C_1, chicken CK-MM; C_2, chicken unidentified.

ings identified and confirmed the observations on the electrophoregrams. The differences in mobility and intensity of the CK-MM bands (with the exception of beef/pork mixture) appear to be a useful tool for identifying each species when stained for general proteins. CK isozyme bands detected by their enzymatic activities were not stable enough to quantitate each species in binary meat mixtures. Although this method did not separate as many bands as isoelectric focusing does, bands were highly reproducible and very easy to quantitate due to rather simple and clear densitometric tracings. Moreover, the clinical system adopted for the species identification is readily available, inexpensive, utilizes prepared gels and requires less than 4 hr to run the test. Additional studies with a larger number of samples are needed in order to confirm quantitative identification of meat species in binary mixtures. The electrophoretic pattern of turkey sarcoplasmic proteins, which shows a distinct and sharp CK-MM band, is particularly promising, in view of the growing consumption of turkey meat.

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—Continued on page 741

Binding of Meat Pieces: Influence of Some Processing Factors on Binding Strength and Cooking Losses

J. J. MACFARLANE, R. H. TURNER and P. N. JONES

ABSTRACT

Evacuation of the chamber of the press used to form flaked beef into a restructured meat product (patty) did not affect the binding strength of the cooked patty but under some conditions decreased the cooking loss. In studies using patty mixes containing 0–1.0% added sodium chloride, meat binding strength increased with decrease in the temperature of the mix when formed into patties over the temperature range −1° to −5°C. The largest effect generally occurred between −1° and −2°C. However, the effect was only noted in patties that were frozen (−30°C) before being cooked for assessment. With decrease below −1°C of the temperature of patties when pressed, cooking losses increased for the patties without added salt but decreased for those with added salt (0.5% or 1%). Change in the pressure applied to form the patty (in the range 1.4–13.7 MPa) can affect binding strength.

INTRODUCTION

OVER RECENT YEARS much interest has been shown in the processing of relatively low value meat trimmings and tougher cuts of meat into higher value, restructured products resembling steak. Although the procedures used to do this vary in detail, they generally involve a similar sequence of operations, namely, production of suitably sized particles or slices of meat, then mixing these with or without additives such as sodium chloride or polyphosphates and finally forming the mixture into desired shapes. Studies that have been made of these operations have been reviewed recently by Breidenstein (1982), Huffman and Cordray (1982), and Mandigo (1982).

The effect of some of the variables associated with these operations on the binding achieved between meat particles in restructured meat products that have been cooked are not well defined. In particular no information appears to be available about the effect on binding of the temperature of meat particles at the time they are consolidated into a muscle mass. Any effects due to change in this variable are likely to be most pronounced over the range of temperatures that much of the water in meat freezes, i.e, −1° to −5°C. The objectives of the present study were to investigate the effect on the binding strength of, and the cooking loss from, a restructured meat product ('meat patties') of (1) the temperature of meat particles at the time of pressing, (2) the mixing time of the patty mix prior to pressing, (3) the formation of the patty under vacuum, and (4) the pressure applied during patty formation. The patties investigated had relatively low (0–1.0%) added sodium chloride (salt) content. Such low levels of salt have been recommended for restructured products (Mandigo, 1982).

MATERIALS & METHODS

THE EXPERIMENTAL PROCEDURE was varied between experiments, as outlined below, to suit the aims of the particular investigation. Experiments were replicated three times (using muscles from different carcasses) except for Exp. 3, which was replicated four times.

Experiment 1 — Mixing time, evacuation before pressing

Frozen (−30°C) beef forequarter muscles trimmed of fat were stored at −1° to −2°C for 3 to 7 days, then cut to give pieces measuring approximately 3 × 3 × 6 cm. These pieces were flaked using a Comitrol Flaker Model 3600 (Urschel Laboratories, Inc., Valparaiso, IN) equipped with a 2-K-060510 head. The flaked meat was used to prepare the seven mixes, each of 2 kg, with composition as indicated in Table 1. Salt, when included, was first dissolved in the water to be added to the mix. Mixing using a Crypto Rotabowl mixer (Crypto, Ltd., London, England) was done in a cold room controlled at 5 ± 0.5°C.

Patties (10 cm diameter, approximately 1.3 cm thick) were formed using a hydraulic press equipped with a chamber that could be evacuated (Fig. 1). Six 100g samples from each mix were pressed at 13.7 MPa for 5 sec. Three of these were pressed without first evacuating the press chamber, while for the remaining three the chamber was evacuated for 10 sec, reducing the pressure to 0.068 Pa immediately before pressing. Patties were carefully removed from the press, individually packaged in polyethylene bags and then stored for 1 to 2 days in a freezer at −30°C before assessment.

Experiment 2 — Temperature and shape before freezing and freezing conditions

Frozen beef forequarter muscles trimmed of fat were held at −1°C to −2°C for 2 to 7 days, then flaked using a Comitrol machine as described for Experiment 1. Two 5 kg batches of meat flakes were mixed for 5 min in a Crypto Rotabowl mixer in a cold room at 5°C. Each batch was divided into 30 × 100g samples, 15 of which were gently pressed into a disc shape, 10 cm diam, using a hand press (Omas (Aust.) Pty. Ltd.), and the remaining 15 were gently formed by hand into spheres.

From one batch, three disc and three spherical-shaped samples were placed in each of five chambers that were temperature-controlled to give −1°, −2°, −3°, −4°, and −5°C. After 24 hr, these samples, herein referred to as 'temperature adjusted', were pressed at 13.7 MPa for 5 sec, then stored at −30°C. For the above 'pressing' operation, the press was located in a room temperature-controlled at −5 ± 0.5°C, in which room the temperature-controlled chambers were also located. As required, samples were quickly transferred from the temperature-controlled chambers to the press and pressed immediately so that pressure was applied to the patty while it was at the desired temperature. Patties were carefully removed from the press, then packaged in polyethylene bags and stored at −30°C for 2 to 4 days.

The 30 samples from the other batch were stored at −30°C for 24 hr and then three each of the disc and the spherical samples were tempered for 24 hr in each of the chambers controlled at −1°, −2°, −3°, −4°, and −5°C. These samples were pressed and stored in the same way as the 'temperature adjusted' samples.

Experiment 3 — Cooking from the frozen and the non (or partially) frozen state

Three frozen (−30°C) beef chucks trimmed of fat were stored in a room at −1° to −2°C for 6 days to temper, then individually flaked in the Comitrol. A 2 kg sample of flakes was taken from each chuck and mixed separately for 5 min in the Crypto Rotabowl mixer. Twelve 100g samples from each mix were each gently pressed into a disc shape using the hand press; six of these were stored at −1°C and the rest at −2°C for 24 hr. Each sample was pressed at 13.4 MPA for 5 sec and packaged in a polyethylene bag. Three patties from each temperature treatment were cooked for 15 min in a water-bath tem-

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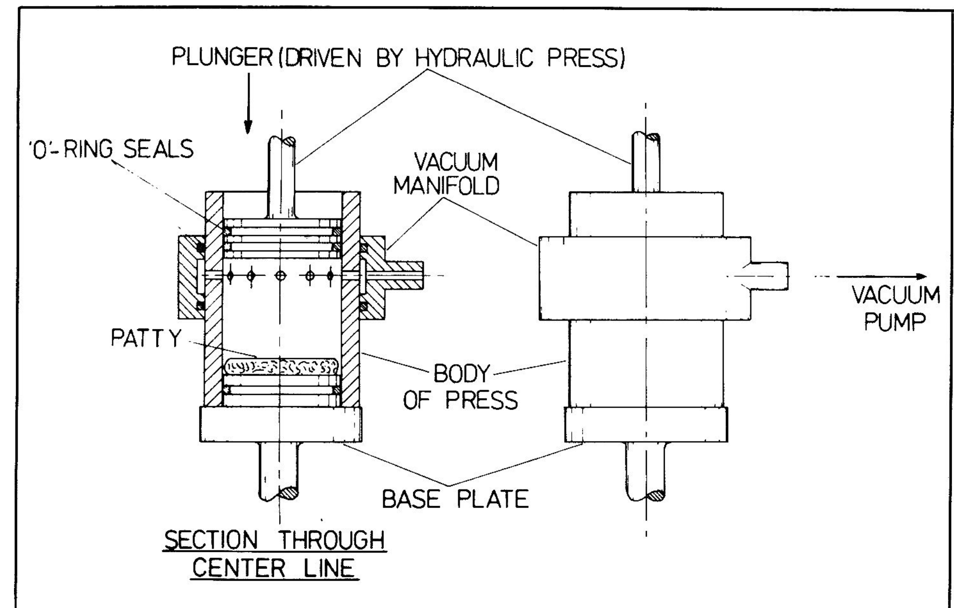

Fig. 1—Press used to form patties. Diameter of the chamber of the press: 10 cm.

perature controlled at 70°C. The remaining three samples from each temperature treatment were stored at −30°C for 24 hr, then cooked from the frozen state for 20 min at 70°C.

Experiment 4 — Temperature before pressing and NaCl concentration

Chucks, trimmed of fat, from beef carcasses 1 day postmortem were frozen at −30°C for 2 to 8 days, stored at −1° to −2°C for 3 days and flaked as for Experiment 1. This meat was used to prepare 3 × 3 kg mixes (mixed for 5 min at 5°C in the Crypto Rotabowl mixer) each containing 5% added water and 0, 0.5 and 1.0% added NaCl, respectively. Salt, when included, was first dissolved in the water to be added to the mix. Each mix was divided into 15 × 100g samples which were lightly pressed into discs using the hand press and stored at −30°C for 1 day. Samples in groups of three were then stored at −1°, −2°, −3°, −4°, and −5°C, and pressed following the same procedures described for the 'temperature adjusted' samples of Experiment 2.

Experiment 5 — Salt concentration, pressing force and pressing time

Twelve 100g samples from each of the mixes of different salt concentrations referred to in Experiment 4 were pressed into a disc shape using the hand press and stored at −30°C for 4 days. The frozen samples were stored at −5°C for 4 days; two samples were assigned to each of the pressing treatments of 1.4, 4.4, or 13.7 MPa for 5 or 60 sec. The samples were stored at −30°C for 1 day and then removed for assessment.

Measurement of temperature

Thin copper-constantan thermocouples were used. Meat temperatures before and after flaking and before and after mixing were measured to confirm that only small temperature rises (<0.5°C) occurred during these operations. When salt was present, mixing resulted in a small decrease of temperature (−0.8°C at 1% added salt). The temperature of each patty mix was measured by carefully inserting a thermocouple into the central region of the mix. In experiments 2-5 where the mix may be partially frozen, the temperature of each patty portion taken from the mix was measured immediately before pressing. The internal temperature attained during cooking of patties was also measured by means of a thermocouple.

Estimation of pressure applied to patties

Measurements were made of the pressure developed in the chamber, when filled with water, at appropriate pressure-settings of the hydraulic press. To enable the pressure of the water in the chamber to be measured, a pressure gauge was fitted to a small hole drilled in the side of the chamber. The pressure gauge was removed, and the hole sealed, when patties were to be pressed.

Cooking method

Unless stated otherwise, samples in tight-fitting water-tight polyethylene bags were cooked from the frozen state (−30°C) for 20 min submerged in a water bath temperature controlled at 70 ± 0.2°C (internal temperature attained by patties: 68.5°C approximately). It was considered that cooking in a water bath gave greater assurance of uniformity of cooking conditions than given by other methods such as grilling of frying). As soon as samples were removed from the cooking bath, they were cooled to < 30°C in running tap water. Cooking loss was determined as described by Bouton et al. (1971).

Measurement of binding strength

Each cooked patty was cut into two samples measuring 5 × 2.5 × 1 cm. Samples were clamped at each end by special holders similar to those described by Macfarlane et al. (1984). These had been fitted to an Instron Universal Testing Machine so that the holders were 2 mm apart. Binding strength, measured as peak force from the stress-strain curve, was determined by pulling sample holders apart using a crosshead speed of 100 mm min^{-1}.

Statistical methods

The experiments were analyzed as complete factorial designs or split plot designs. In all cases binding strength and cooking loss were the measurements analysed by analysis of variance.

RESULTS & DISCUSSION

Mixing time, evacuation before pressing (Experiment 1)

After they were cooked, patties formed without evacuation before pressing had small cavities in the regions of the junctions between meat particles. It was thought that if these could be eliminated by forming the patty in an evacuated chamber, the binding strength of patties might be improved and variability in binding strength reduced. However, evacuation did not affect binding strength, and no interactions were found between the evacuation and mixing time, salt combinations (Tables 1 and 2). Thus, at least under the conditions employed here, cavities were of little or no consequence with respect to the tensile measurements and the evacuation procedure was not used in the remaining experiments. For cooking loss, there was significant interaction between evacuation and the two salt levels tested, namely 0 and 0.5% added salt (Table 2). In the case of nil added salt, evacuation appeared to have little or no

Table 1—Influence of mixing time and evacuation of the patty press immediately prior to patty formation on mean value for the binding strengths of and the cooking losses from patties

Product description			Binding strength (kg)[a]		Cooking loss (%)[a]	
Added NaCl (%)[b]	Added water (%)[b]	Mixing time (min)	Not evacuated	Evacuated	Not evacuated	Evacuated
0	0	20	0.272	0.302	19.3	16.3
0	10	5	0.140	0.151	24.2	24.3
0	"	10	0.180	0.221	24.1	25.5
0	"	20	0.292	0.284	25.1	23.9
0.5	"	5	0.188	0.208	24.6	21.5
0.5	"	10	0.275	0.269	23.5	20.0
0.5	"	20	0.352	0.302	20.5	18.0

[a] Least significant differences, P = 0.05:Binding strength 0.070 kg; Cooking loss 2.7%.
[b] Amount added expressed as a % of total mix.

Table 2—Analysis of variance for the data of the experiment reported in Table 1

		Binding strength			Cooking loss		
Source	df	MS	F	Sig. level[a]	MS	F	Sig. level[a]
Replicates	2	0.003819			100.556		
Added water (W)	1	0.014235	6.3	*	125.730	51.7	***
Salt (S)	1	0.024964	11.1	**	95.388	39.2	***
Mixing time (T)	2	0.053294	23.7	***	12.054	5.0	*
Lin	1	0.104262	46.3	***	23.964	9.8	**
Quad	1	0.002325	1.0	NS	0.143	0.1	NS
S × T	2	0.000869	0.4	NS	10.534	4.3	NS
Deviations × Lin	1	0.000325	0.1	NS	20.846	8.6	*
Deviations × Quad	1	0.001412	0.6	NS	0.221	0.1	NS
Error (1)	12	0.002253			2.433		
Evacuation (E)	1	0.000688	0.5	NS	24.687	7.9	*
W × E	1	0.001781	1.3	NS	1.991	0.6	NS
S × E	1	0.001995	1.4	NS	24.668	7.9	*
T × E	2	0.002302	1.7	NS	0.567	0.2	NS
S × T × E	2	0.000471	0.3	NS	2.450	0.8	NS
Error (2)	14	0.001382			3.121		

[a] *,**,*** significant at P = 0.05, 0.01, 0.001 level respectively; Ns = not significant

Table 3—Effect of freezing patties at −30°C after pressing at −0.7°C or at −2.2°C, on binding strength and cooking loss

	Binding strength (kg) of patty	
	Pressed at −0.7°C	Pressed at −2.2°C
not frozen at −30°C	0.262 (68.5°C)[a]	0.289 (67.5°C)
frozen at −30°C	0.224 (68.2°C)	0.326 (68.5°C)
Least significant difference (P = 0.05) : 0.036 kg		
	Cooking loss (%) of patty	
	Pressed at −0.7°C	Pressed at −2.2°C
not frozen at −30°C	16.16	13.12
frozen at −30°C	16.92	15.97
Least significant difference (P = 0.05) : 1.30%		

[a] The figure in brackets is the temperature attained during cooking.

effect on cooking loss, whereas in that of 0.5% added salt, cooking losses were reduced by evacuation (Table 1). With neither added water nor salt, evacuation also reduced cooking loss. At present no explanation for the variable effect of evacuation on cooking loss is apparent.

A highly significant increase in binding strength occurred with increase in mixing time (Tables 1 and 2), which is consistent with the increase in binding strength with increase in mixing time reported by Pepper and Schmidt (1975). However, concomitant with the increased binding strength achieved with increased mixing time, a loss in the fibrous appearance of the product was noticed. Therefore, if a steak-like appearance is desired, mixing should not be prolonged unnecessarily. In subsequent experiments, a mixing time of 5 min was used. For cooking losses there was a significant linear component of the interaction between salt (levels 0, 0.5) and time (Table 2). The cooking losses at nil added salt did not significantly change with time, but at 0.5% added salt they decreased linearly (Table 1 and 2). The latter decrease presumably resulted from increased penetration of the myofibrillar structure by salt with increased mixing.

Temperature and shape when inserted into the press (Experiments 2 and 3)

To simplify discussion of the results, patty temperature here is stated to the nearest degree Celsius, which in all cases coincides with the temperature stated for the particular chamber used to adjust patty temperature. The actual measured temperatures of patties are as indicated in Fig. 2 and 3 and Tables 3 and 4.

The binding strength of patties was affected by the temperature of the mixed meat flakes when pressed (Fig. 2a). When the mix, as inserted into the press, was already of a disc shape that approximated the shape of the patty formed by the press (solid symbols, Fig. 2a), a marked increase in binding strength occurred as the temperature of the patty when pressed was decreased from −1° to −2°C. By −3°C a maximum value had been reached which was maintained at −4° and −5°C. Provided super-cooling did not occur, ice formation would be initiated between −1° and −2°C. Therefore, it is possible that in patties at −2°C or lower, thawing and refreezing of ice during the pressing operation helped to increase binding strength. The presence of ice may also render the patties pressed at or below −2°C less susceptible to mechanical damage during subsequent handling than those pressed at −1°C. To eliminate as much as possible the influence of this factor on the results, patties were handled very carefully after they were pressed.

The data for Fig. 2 were obtained from patties that were frozen at −30°C after they were pressed (patties were frozen to achieve good stability during storage and to simplify achievement of similar cooking condition for all samples, regardless of temperature differences when they were pressed). Therefore, the difference between the results at −1°C and

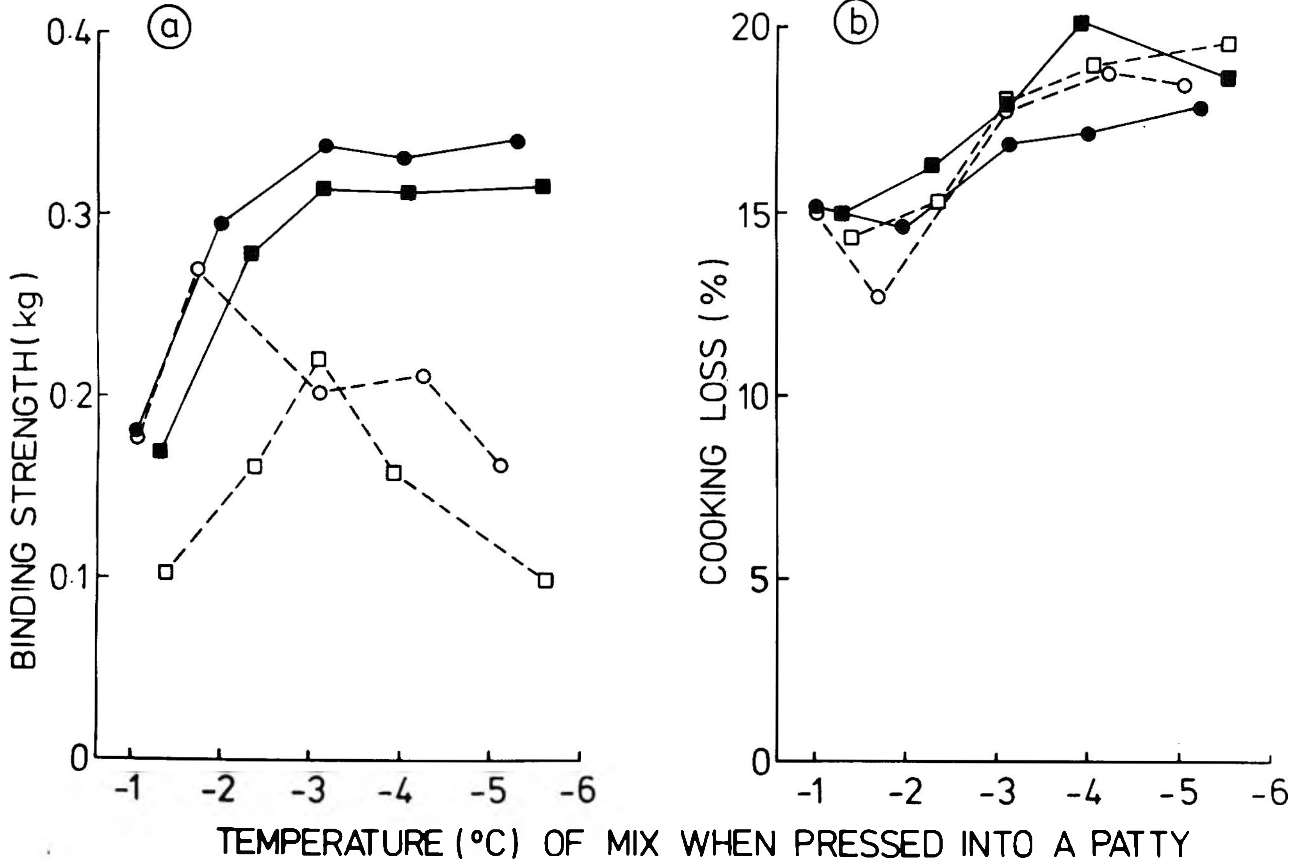

Fig. 2—Effect of the temperature of the patty mix at the time of pressing into a patty on binding strength (Fig. 2a) and cooking loss (Fig. 2b). Samples when loaded into the press were either of a spherical or a disc shape. ● Disc shape initially, temperature adjusted downwards from −1°C; ■ Disc shape initially, frozen at −30°C then temperature adjusted upwards; ○ Spherical shape initially, temperature adjusted downwards from −1°C; □ Spherical shape initially, frozen at −30°C then the temperature adjusted upwards. Least significant differences, P = 0.05. Binding strength: 0.081 kg; Cooking loss: 2.2%.

Table 4—Effect on binding strength and cooking loss of change in the pressure applied to patties at −5.1°C and containing various concentrations of added salt

Salt conc[a] (%)	Pressure applied (MPa)	Binding[b] strength (kg)	Cooking[b] loss (%)
0	1.4	0.169	23.3
	4.4	0.204	23.3
	13.7	0.224	24.3
0.5	1.4	0.309	20.6
	4.4	0.411	21.0
	13.7	0.371	19.8
1.0	1.4	0.473	14.8
	4.4	0.479	15.5
	13.7	0.430	15.5

[a] Amount of added sodium chloride, as a percentage of total mix.
[b] Least significant differences, P = 0.05; Binding strength 0.065 kg (0.030 kg for same salt concentration); Cooking loss 2.1% (0.8% for same salt concentration).

−2°C may have been due to the freezing step as the patties initially at −1°C had no ice present whereas those at −2°C had some present. To investigate this possibility, patties were pressed at −1° and −2°C and half of them kept at the temperature at which they were pressed, and the remainder frozen at −30°C. In order to achieve similar temperatures in the cooked patties (Table 3), the frozen (−30°C) patties were cooked for 20 min at 70°C, whereas the others were cooked for 15 min. From the results presented in Table 3, it can be seen that no significant differences in binding strength between patties pressed at −1° and at −2°C were found if patties did not receive the −30°C freezing treatment. Subsequent freezing of patties decreased the binding strength of those pressed at −1°C ($P<0.05$), but increased it for those pressed at −2°C ($P<0.05$). This indicates that the freezing of samples after they were pressed was responsible for the differences in binding strength between samples pressed at −1°C and at −2°C.

For the samples that were not frozen at −30°C, in contrast to the above-noted lack of temperature during pressing effect on binding strength, cooking losses were lower in samples pressed at −2.2°C than at −0.7°C (Table 3). This difference, at least in part, may be due to the small difference between these samples in the temperature attained during cooking. Freezing at −30°C increased the cooking loss from the samples pressed at −2.2°C, so that cooking losses were not significantly different from those of the samples pressed at −0.7°C, then either frozen or not frozen.

Because the temperature of meat when flaked affected flake characteristics, the meat was flaked at about the same temperature (−1.5° to −1.7°C). A consequence of this was that to achieve a range of flake-mix temperatures, flakes after mixing were divided into portions and the temperature of each portion was adjusted to the desired temperatures. With decrease in the temperature of meat below the freezing point (approximately −1.5°C), the proportion of water in the frozen state increased, so that at −5°C about 84% of the freezable water in meat was frozen (Morley, 1974). Therefore, when the temperature of meat flakes was adjusted within the range −1.5° to about −5°C, much consolidation of flaked material could occur as a result of ice formation.

Mandigo (1982), presumably in reference to a partially frozen mass of meat particles formed into the shape of a log that was to be reshaped, commented that during pressing steps it is important that "the product not be forced to flow to a great extent." He stated that this is because "at this temperature the meat will set up shear-lines in the steaks, which will be then

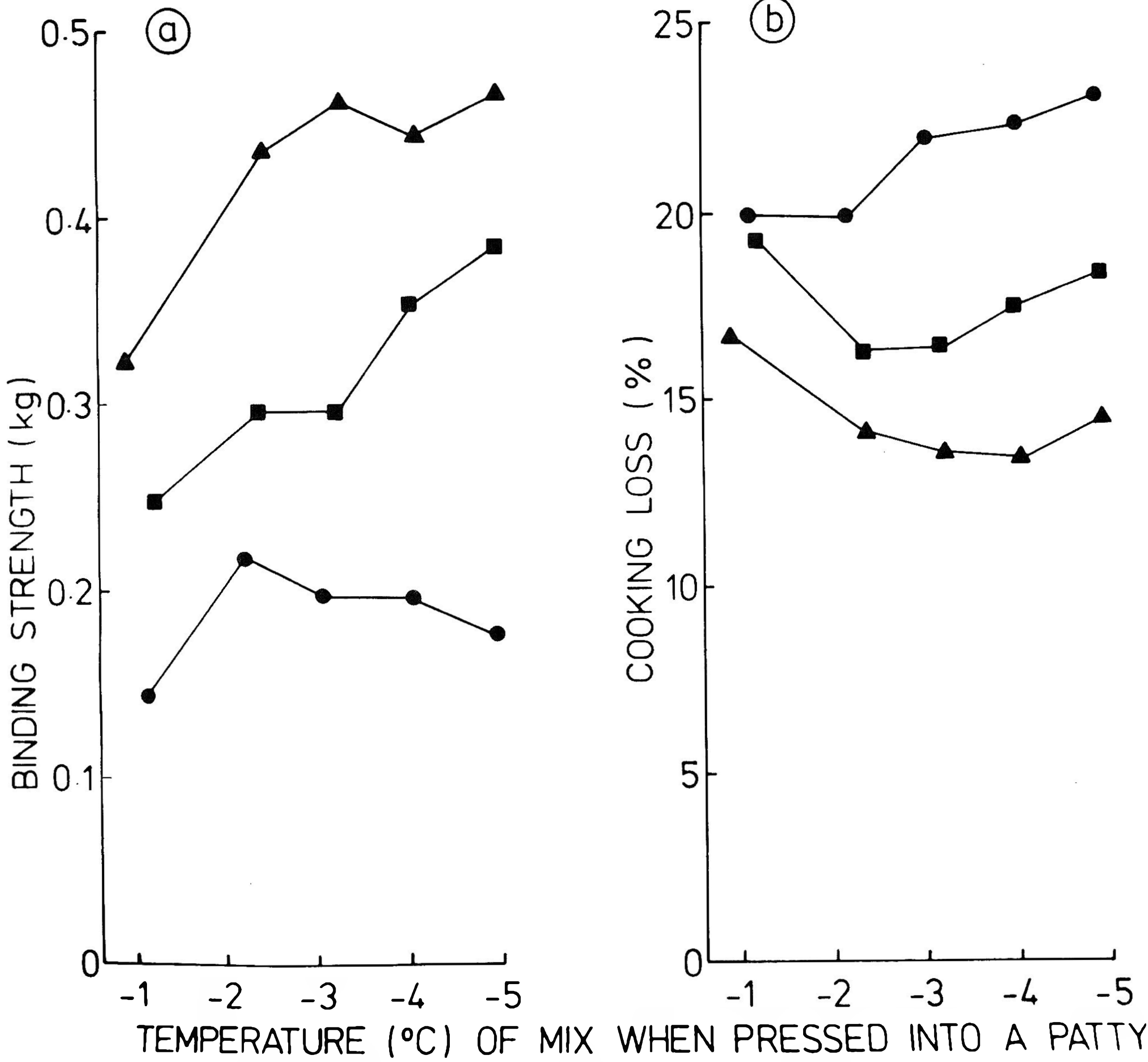

Fig. 3—Influence of salt addition on the relationship between the temperature of the patty mix at the time of pressing into a patty and binding strength (Fig. 3a) and cooking loss (Fig. 3b). ● no added salt; ■ 0.5% added salt; ▲ 1.0% added salt. Least significant differences, P = 0.05; Binding strength: 0.082 kg (0.063 kg, same salt level); Cooking loss: 2.4% (1.4%, same salt level).

more prone to breaking and coming apart during subsequent handling and cooking." In the present investigation this factor could be important when a frozen or partially frozen portion of flaked meat was reshaped in the press. The results of an experiment in which samples initially of either spherical form or disc form were reshaped in the press are given in Fig. 2a and b. The major physical structural rearrangement that occurred when patties of spherical form were pressed decreased the binding strength (Fig. 2a). However, binding strength of these samples increased with the initial decrease of pressing temperature below − 1°C, then decreased as the temperature was lowered further and ice formation was more extensive. The binding strength of samples that were initially of spherical form depended upon whether their temperature before pressing had been adjusted downwards from − 1°C, or upwards from − 30°C. Binding strength of samples that were initially of disc form was not affected by the direction of temperature adjustment.

Cooking losses increased as the temperature at which the patty was pressed was decreased from − 1°C to − 3°C approximately regardless of the shape of the patty mix prior to pressing (Fig. 2b).

Addition of salt (Experiment 4)

Addition of salt to patties, besides promoting swelling and solubilization of myofibrillar proteins, also lowered the freezing point of the aqueous phase (by about 0.6°C for the addition of 1% salt to pure water). In patties with 1% added NaCl, increase in binding strength ($P<0.05$) occurred when the temperature at which the patty was pressed was decreased from − 1°C to − 2°C (Fig. 3). Little further change was found with decrease of temperature to − 5°C. In patties with 0.5% added NaCl, the increase in binding strength values was not significant when the temperature at the time of pressing patties was decreased from − 1°C to − 2°C. However, the values increased progressively with further decrease in temperature so that increases were significant by − 4°C. Differences in changes in binding strength with change of temperature apparent from a comparison of Fig. 2a and 3a may be due to the addition of 5% water to the patties represented in Fig. 3. This addition was made to facilitate the dispersion of added NaCl through these patties.

Cooking losses for patties without added salt increased with decrease of temperature when pressed in the range − 1° to

Table 5—Analysis of variance for the data of the experiment reported in Table 4

Source	df	Binding strength MS	F	Sig. level[a]	Cooking loss MS	F	Sig. level[a]
Replicates	2	0.068172			10.7089		
Salt concentration (S)	2	0.314486	42.4	**	321.2067	35.2	***
Lin	1	0.614656	82.9	***	630.0100	69.0	***
Quad	1	0.014315	1.9	NS	12.4033	1.4	NS
Error (1)	4	0.007411			9.1256		
Pressure applied (P)	2	0.010512	10.6	***	0.7439	1.5	NS
Lin	1	0.001060	1.1	NS	0.4954	1.0	NS
Quad	1	0.019964	20.2	***	0.9924	2.0	NS
Time (T)	1	0.000521	0.5	NS	0.7585	1.5	NS
S × P	4	0.007411	7.5	***	2.1614	4.3	**
Lin × Lin	1	0.014890	15.1	***	0.4147	0.8	NS
Quad × Lin	1	0.002154	2.2	NS	6.8100	13.6	***
Lin × Quad	1	0.000120	0.1	NS	1.1870	2.4	NS
Quad × Quad	1	0.012478	12.6	**	0.2339	0.5	NS
S × T	2	0.000459	0.5	NS	0.0007	0	NS
P × T	2	0.000163	0.2	NS	0.0080	0	NS
S × P × T	4	0.002274	2.3	NS	0.5677	1.1	NS
Error (2)	30	0.000987			0.5002		

[a] *,**,*** significant at P = 0.01, 0.001 level respectively; NS = not significant.

−3°C (Fig. 2b and 3b). In contrast, in patties with 1% or 0.5% added salt, cooking losses decreased with decrease of temperature when pressed from −1° to −2°C. Therefore, added salt appeared to overcome the adverse effects on WHC of pressing samples that were partly frozen.

Pressure in patty press (Experiment 5)

The application of very high pressures (150 MPa) to meat patties has been reported to increase binding strength (Macfarlane et al., 1984). However, Costello et al. (1981), in an investigation of the response of restructured steaks to much lower pressures of approximately 1.4, 4.1 and 6.0 MPa, found no differences in cooking characteristics or sensory properties.

The results obtained in the present study for binding strengths and cooking losses for patties formed at various pressures and amounts of added salt are shown in Table 4. Whether pressure was maintained on a patty for 5 or for 60 secs did not significantly affect either of these attributes (Table 5), and consequently in Table 4 means over the two durations of pressure treatment are presented. From Table 5, it can be seen that for binding strength a highly significant quadratic-quadratic interaction occurred between salt concentration and pressure applied. For patties with no added salt, binding strength increased with increase of pressure over the pressures investigated, namely 1.4, 4.4 and 13.7 MPa (Table 4). For patties with 0.5% added salt, increase in binding strength occurred between pressures of 1.4 and 4.4 MPa, whereas for patties with 1.0% added salt, increase of pressure did not improve binding strength. For cooking loss, a very highly significant quadratic-linear interaction occurred between salt concentration and pressure applied. However the changes in the values for cooking loss for the three levels of applied pressure were relatively small compared to those resulting from change in salt level.

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Laboratory and Pilot Scale Recovery of Protein from Mechanically Separated Chicken Residue

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ABSTRACT

Bone residue resulting from mechanical deboning of chicken backs and necks was subjected to alkali extraction followed by acid precipitation to recover food grade protein. A laboratory study of effects of varying die size in the grinding of the starting material, of extraction temperature, and of g-force during centrifugation on composition of process fractions was conducted. Grinding increased ash content, higher temperature reduced fat content, and increasing centrifugal force decreased ash content, of the final products. Effects of grinding, extraction temperature, and centrifuge type were also studied on a pilot scale. Final protein product yield was higher at a 22°C than at a 3–7°C extraction temperature, and less fat was incorporated using a basket centrifuge than with a horizontal decanter centrifuge.

INTRODUCTION

IN THE BUTCHERING PROCESS, after the normal cuts of meat have been fabricated, a certain amount of meat is left firmly attached to the bones. In the past, as much of this meat as possible was removed by tedious hand deboning. To make this process more efficient, mechanical methods for removing the meat from bone have been developed. The first successful machines were developed for fish in the late 1940's; mechanical deboning of poultry began 10–15 years later (IFT Expert Panel on Food Safety and Nutrition, 1979). Mechanical deboning of poultry recovers 50 to 75% of the meat remaining on the bones (Anon., undated), which would otherwise be rendered to animal feed. The recovered mechanically deboned meat is used primarily in emulsified products such as sausages (Froning, 1976, 1981), and the deboner residue is rendered.

It has been suggested that the bone residue from mechanically deboned poultry meat merits greater utilization (Froning, 1976). Bone residue contains 16–20% protein (as-is basis), and although most of this protein is collagen, over 18% is alkali-extractable sarcoplasmic and myofibrillar protein that could be a valuable protein ingredient in processed meat products (Lawrence et al., 1982). Low temperature, aqueous extraction of protein from bone residue would allow the recovery of the protein in its undenatured form with desirable functional properties, as compared to high temperature rendering, solvent extraction, or enzymatic solubilization processes (Jelen et al., 1978). Several investigators have studied the feasibility of recovering residual meat protein from the bones which are obtained after hand or mechanical removal of the meat. Alkaline extraction of protein from mechanically deboned poultry residue has been studied by Young (1976) and Jelen's group (Lawrence, 1981; Lawrence and Jelen, 1982; Jelen et al., 1982) and various means of extraction of proteins from beef boning room wastes have been investigated at Massey University, New Zealand (Duerr and Earle, 1974; Hamilton and Richert, 1976; Jelen et al., 1978, 1979). A patent for a process incorporating alkaline extraction-acid precipitation of protein from bone residues has been issued to Herubel (1982).

Laboratory studies using bone residue obtained from the mechanical separation of meat and bone from poultry and beef carcasses have explored the conditions for the extraction and recovery of functional protein. Alkaline extraction appears to be the best method for solubilizing the protein (Jelen et al., 1979), which is then recovered by acidifying to pH 4.5-5.0. Heat aids precipitation and increases the protein yield, but may adversely affect the functional properties of the product (Jelen et al., 1979).

Several studies on the utilization of the protein which has been recovered from deboner residues by alkaline extraction-acid precipitation have also been conducted. Jelen et al. (1982) found that addition of up to 20% of alkali extracted poultry protein did not significantly alter the quality of luncheon meat made from mechanically separated poultry meat. Caldironi and Ockerman (1982) substituted their alkali extracted protein from beef bones for beef protein in sausages at levels of up to 15% and produced a very acceptable product. Alkali extracted-acid precipitated protein from beef bones was shown to have a good amino acid profile (Golan and Jelen, 1979), while no formation of lysinoalanine, a potentially toxic crosslinked amino acid, was detected under the alkali extraction conditions proposed (Lawrence and Jelen, 1982).

Pilot scale evaluation of the process for extracting protein from the bone residue that results from mechanical deboning has been limited to one study on poultry residues (Lawrence et al., 1982). Transfer of the technology from laboratory scale to pilot scale created problems related to the blockage of flow through pipes between tanks and centrifuges (due to size of bone and skin pieces), a high fat content in the final protein product as compared to laboratory studies, and a high protein loss in the supernatant resulting in a low content of protein in the final product.

Thus, the purpose of this investigation was twofold. The first objective was to investigate, on a laboratory scale, the effects of: (1) die size in the grinding of the starting material, (2) temperature of the extraction-precipitation procedure, and (3) the g-force during centrifugation, on the recovery of the final protein product resulting from the alkaline extraction-acid precipitation processing of the residue from mechanical deboning of chicken backs and necks. Using the information obtained in the laboratory studies, the second objective was to investigate, on a pilot scale, the effects of grinding, extraction temperature, and centrifuge type on the recovery of chicken protein from bone residue.

MATERIALS & METHODS

Materials

Approximately 16 kg mechanically separated chicken residue (MSCR) from the mechanical deboning (Beehive deboner model AUXS 2445, Beehive Machinery, Inc., Sandy, UT) of chicken backs and necks was obtained for laboratory experimentation from a poultry processing plant (Plains Poultry, Wynyard, Sk.), mixed thoroughly, subdivided into 500 or 1,000-g lots, and stored at −17°C until the protein extraction and recovery procedure was conducted. For pilot scale experiments, the MSCR was obtained from the same poultry processing

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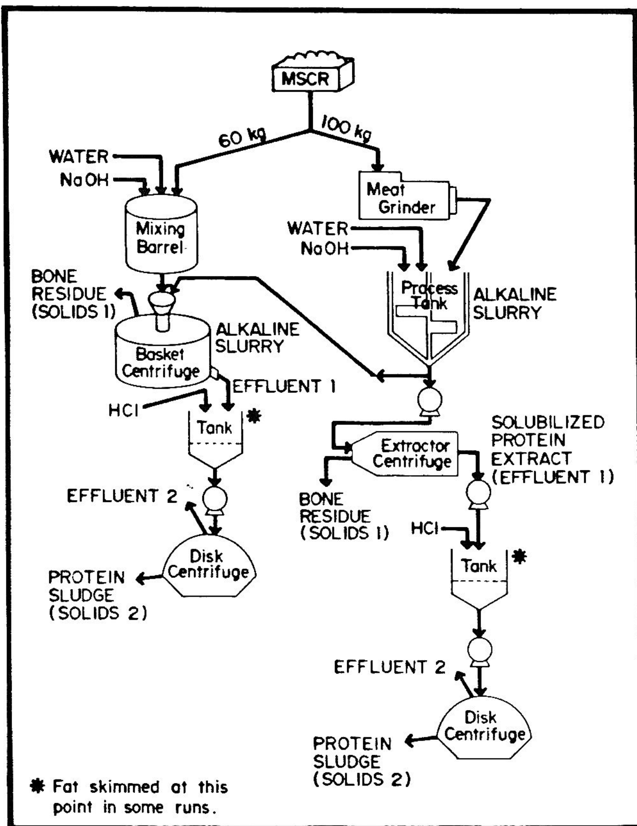

Fig 1—Pilot scale procedure for alkaline extraction-acid precipitation of protein from mechanically separated chicken residue.

plant; between 60 and 100 kg MSCR per batch were used in the pilot scale experiments.

Laboratory experiments

General extraction-precipitation method. The protein extraction procedure was adapted from the conditions determined by Jelen's work (Jelen et al., 1979; Lawrence et al., 1982). The residue material was mixed with tap water (ratio of 1:1.25 by weight) and sufficient 20%(w/v) aqueous sodium hydroxide was added to raise the pH to 10.5. The alkaline extraction of protein was continued with stirring for 30 min, with the metering in of additional base as necessary to maintain the pH. Then the slurry was centrifuged. Protein in the fat-skimmed supernatant (supernatant 1) was precipitated by the addition of 3N HCl to pH 5.3, and recovered by centrifugation (precipitate 2). Precipitate 1 and supernatant 2 were sampled and discarded. The weight and proximate analysis were determined for each fraction so that material balances could be calculated. This general procedure was varied as follows to assess the effects of grinding, temperature, and centrifugal force on extraction and recovery.

Control experiments: MSCR, as received from the poultry processing plant, was extracted at 22°C, with g-forces at 4300 and 9400 for the first and second centrifugation steps, respectively.

Grinding experiments: MSCR was ground through either a 0.63 or 0.32 cm die prior to extraction, then extracted at 22°C with g-forces at 4300 and 9400 for the first and second centrifugation steps, respectively.

Temperature experiments: MSCR was ground through a 0.63 cm die, then extracted at either 10° or 32°C, with g-forces at 4300 and 9400 for the first and second centrifugation steps, respectively.

Centrifugal force experiments: MSCR was ground through a 0.63 cm die, then extracted at 22°C with g-force at 1800, 4300, or 14,000 for the first centrifugation and at 1800, 9400 or 14,000 for the second centrifugation (while the first centrifugation was held constant at 4300 x *g*).

Pilot plant experiments

The procedure was essentially the same as that used for laboratory experiments, but adapted to pilot plant equipment and conditions. The use of two types of centrifuges for recovery of solubilized protein was investigated. A flow diagram is shown in Fig. 1. Data were collected in six separate pilot scale extractions conducted on three days. For each extraction, material weight and proximate analysis were measured for each fraction so that material balances could be calculated.

Grinding. For some experiments, the MSCR was ground through a 0.32 cm diameter die (1500 Hobart meat grinder) prior to extraction, so that the slurry could be pumped through a horizontal decanter centrifuge without plugging. In other experiments, the MSCR was used as received from the processing plant and the slurry was manually bucketed to the basket centrifuge.

Protein solubilization. The MSCR was added to water at either room temperature (18°–22°C) or prechilled (0–4°C) containing 0.018 kg 20%(w/w) NaOH per kg MSCR (final slurry base concentration was approximately 0.16% (w/w) or 0.04 M NaOH). The alkaline slurry was mixed for 30 min in a process tank or mixing barrel, with base added as necessary to maintain a pH of 10.1 to 10.5. In the

Table 1—Level of significance for the effects of various treatments (grinding, temperature, g-force) on the fractionation of materials and the composition of the fractions recovered during laboratory scale preparation of protein from mechanically separated chicken residue

	Level of significance		
Fraction and assay	Grinding (F-value)	Temperature (F-value)	G-force (F-value)
Starting material			
Moisture, %, as-is basis	NS (0.91)	NS (4.89)	-[d]
Protein, %, d.b.[g]	NS[b] (0.40)	NS (2.07)	-[d]
Fat, %, d.b.	*[b] (7.90)	NS (0.25)	-[d]
Ash, % d.b.	NS[b] (4.61)	NS (1.09)	-[d]
Precipitate 1			
Percentage wet wt[a]	* (6.94)	NS (3.52)	** (10.9)
Percentage dry matter wt[a]	* (8.00)	NS (2.57)	NS (2.35)
Percentage protein wt[a]	NS[b] (2.09)	NS (1.16)	NS (0.63)
Moisture, %, as-is basis	NS (1.16)	NS (1.61)	* (6.32)
Protein, %, d.b.	NS[b] (0.11)	NS (1.04)	NS (3.82)
Fat, %, d.b.	NS[b] (0.23)	* (10.5)	NS (0.33)
Ash, %, d.b.	NS[b] (4.87)	* (5.40)	NS (0.58)
Supernatant 1			
Percentage wet wt	* (5.59)	* (5.56)	NS (3.56)
Percentage dry matter wt	* (7.87)	* (5.25)	NS (0.82)
Percentage protein wt	*[b] (5.68)	NS (4.60)	NS (0.70)
Moisture, %, as-is basis	NS (1.59)	NS (1.90)	NS (0.93)
Protein, %, d.b.	NS[b] (2.68)	NS (2.80)	NS (2.00)
Fat, %, d.b.	*[b,c] (12.47)	***[b] (45.7)	NS (1.05)
Ash, %, d.b.	NS[b,c] (0.90)	NS[b] (2.98)	NS (0.30)
Precipitate 2			
Percentage wet wt	NS (3.31)	NS (5.13)	*** (19.4)
Percentage dry matter wt	NS (0.73)	* (6.83)	NS (0.94)
Percentage protein wt	NS[b] (2.80)	* (6.83)	NS (0.23)
Moisture, %, as-is basis	NS (5.05)	NS (0.91)	*** (39.0)
Protein, %, d.b.	NS[b] (2.94)	NS (5.09)	NS (0.01)
Fat, %, d.b.	*[b] (5.74)	* (7.67)	NS (1.49)
Ash, %, d.b.	***[b] (18.6)	NS (1.05)	***[b] (31.1)
Supernatant 2			
Percentage wet wt	NS (4.60)	NS (0.04)	*** (19.2)
Percentage dry matter wt	NS (0.16)	NS (3.34)	NS (0.40)
Percentage protein wt	NS[b] (1.83)	* (7.04)	NS (0.41)
Moisture, %, as-is basis	NS (0.71)	* (8.83)	NS (0.06)
Protein, %, d.b.	NS[b] (1.98)	NS (3.49)	NS (0.12)
Fat, %, d.b.	NS[c] (0.86)	* (6.23)	-[e]
Ash, %, d.b.	NS[c] (0.89)	** (12.1)	NS[f] (0.03)

[a] Percentage wet weight, percentage dry matter weight, and percentage protein weight refer to the percentage of the total amount of that component originally present in the MSCR which was measured in the fraction.
[b] Missing values in these treatments were estimated by averaging remaining replications.
[c] Parameter measured only for two treatments (0.32 cm and 0.63 cm), thus $F_{1,4}$ used to assess significance.
[d] Starting material composition not measured for each replication in g-force experiments.
[e] Fat composition not measured due to insufficient sample material.
[f] Only two replications (instead of three), thus $F_{2,3}$ used to assess significance.
[g] d.b. Indicates dry weight basis
* Indicates significance at 0.05 level.
** Indicates significance at 0.01 level.
*** Indicates significance at 0.005 level.
NS Indicates not significant.

Table 2—Means of the significant treatment effects of grinding on the fractionation of materials and on the composition of fractions during laboratory scale preparation of protein from mechanically separated chicken residue[a,b]

	Treatment		
Fraction and assay	Unground	0.63 cm die	0.32 cm die
Starting material			
Fat, %, d.b.	27.1 ± 0.2a	22.9 ± 2.1b	20.7 ± 2.8b
Precipitate 1			
Percentage wet wt[c]	54.5 ± 6.4a	44.2 ± 0.5b	45.4 ± 0.6b
Percentage dry matter wt[c]	85.0 ± 2.8a	78.3 ± 3.1b	76.1 ± 2.7b
Supernatant 1			
Percentage wet wt	38.4 ± 7.3b	50.9 ± 2.3a	48.0 ± 3.3a
Percentage dry matter wt	8.9 ± 0.3b	13.9 ± 2.2a	14.5 ± 2.5a
Percentage protein wt[c]	13.7 ± 0.1b	17.1 ± 2.3a	17.1 ± 0.9a
Fat, %, d.b.	-[d]	33.2 ± 0.9a	19.9 ± 6.0b
Precipitate 2			
Fat, %, d.b.	31.5 ± 1.9a	17.6 ± 5.4b	17.1 ± 8.3b
Ash, %, d.b.	1.6 ± 0.0a	4.3 ± 0.6b	4.2 ± 0.9b

[a] Means ± standard deviation.
[b] Means within a row (fraction and assay grouping) followed by different letters are significantly different at the 5% level.
[c] Percentage wet weight, percentage dry matter weight, and percentage protein weight refer to the percentage of the total amount of that component originally present in the MSCR slurry which was measured in the fraction.
[d] Insufficient sample available for analysis.

Table 3—Means of the significant treatment effects of extraction temperature on the fractionation of materials and on the composition of fractions during laboratory scale preparation of protein from mechanically separated chicken residue[a,b]

	Treatment		
Fraction and assay	10°C	22°C	32°C
Precipitate 1			
Fat, %, d.b.	20.7 ± 1.7a	14.4 ± 2.0b	20.4 ± 2.0a
Ash, %, d.b.	29.1 ± 0.5b	32.1 ± 1.9a	30.2 ± 0.2ab
Supernatant 1			
Percentage wet wt[c]	43.7 ± 3.5b	50.9 ± 2.3a	47.3 ± 1.9ab
Percentage dry matter wt[c]	8.5 ± 0.7b	13.9 ± 2.2a	11.7 ± 2.7ab
Fat, %, d.b.	8.1 ± 2.4b	32.2 ± 0.9a	9.9 ± 5.4b
Precipitate 2			
Percentage dry matter wt	6.3 ± 0.0b	9.2 ± 1.9a	6.4 ± 0.3b
Percentage protein wt[c]	10.0 ± 0.4b	13.5 ± 1.9a	10.9 ± 0.7b
Fat, %, d.b.	12.3 ± 2.8ab	17.6 ± 5.7a	5.9 ± 0.3b
Supernatant 2			
Percentage protein wt	2.5 ± 0.1b	3.2 ± 0.4ab	3.7 ± 0.6a
Moisture, %, as-is basis	98.8 ± 0.1a	98.5 ± 0.1b	98.4 ± 0.1b
Fat, %, d.b.	2.9 ± 0.1b	5.2 ± 0.6a	3.6 ± 1.3ab
Ash, %, d.b.	41.9 ± 2.0a	34.7 ± 2.4b	34.9 ± 1.6b

[a] Means ± standard deviation.
[b] Means within a row (fraction and assay grouping) followed by different letters are significantly different at the 5% level.
[c] Percentage wet weight, percentage dry matter weight, and percentage protein weight refer to the percentage of the total amount of that component originally present in the MSCR slurry, which was measured in the fraction.

chilled water extraction, the temperature increased to 3–7°C during the 30 min extraction period.

Recovery of solubilized protein. The alkaline slurry was either pumped to a horizontal 6 × 12 inch decanter centrifuge (Bird Machine Co., Walpole, MA) or bucketed to a perforated bowl, Tolhurst basket centrifuge (Ametek Process Equipment, East Moline, IL) for separation of solubilized protein and residue. The separated effluent 1 (termed supernatant 1 in the laboratory scale experiments) was cooled in a heat exchanger in some experiments. Fat was skimmed from the surface of the centrifuge effluent when possible. The solids 1 (precipitate 1 in laboratory experiments) was discarded.

Recovery of precipitated protein. Effluent 1 was acidified to pH 5.3 with 3N HCl, then pumped to a desludging disk centrifuge (Westfalia Model SA 7-06) for recovery of protein sludge (solids 2, also called alkaline extracted chicken protein or AECP; precipitate 2 in the laboratory experiments).

Table 4—Means of the significant treatment effects of varying centrifugal force during extraction on the fractionation of materials and on the composition of fractions during laboratory scale preparation of protein from mechanically separated chicken residue[a,b]

	Treatment		
Fraction and assay	1800 × g	4300 or 9400 × g	14,000 × g
Precipitate 1			
Percentage wet wt[c]	56.3 ± 2.5a	53.9 ± 1.2a	48.9 ± 2.0b
Moisture, %, as-is basis	69.2 ± 0.4a	69.3 ± 0.6a	68.2 ± 0.2b
Precipitate 2			
Percentage wet wt	27.2 ± 1.8a	24.4 ± 2.5a	17.3 ± 1.7b
Moisture, %, as-is basis	88.4 ± 0.6a	87.0 ± 0.9a	82.6 ± 1.0b
Ash, %, d.b.	4.6 ± 0.3a	3.9 ± 0.2b	3.1 ± 0.2c
Supernatant 2			
Percentage wet wt	71.7 ± 2.3b	74.8 ± 2.2b	82.0 ± 1.8a

[a] Mean ± standard deviation.
[b] Means within a row (fraction and assay grouping) followed by different letters are significantly different at the 5% level.
[c] Percentage wet weight refers to the percentage of the total amount of the original MSCR slurry weight, which was measured in that fraction.

Starting material composition

Because of the variability of composition observed in shipments of MSCR received for the pilot scale extractions, a 500 g sample of MSCR was collected from each shift at Plains Poultry (Wynyard, SK) over a 2 1/2 month period when bone material was being processed through the Beehive deboner. In all, 35 samples were taken and the source of feed to the deboner recorded; eight were from chicken necks, ten from chicken backs, and thirteen from backs and necks. (Both necks and backs had attached skin). Four samples were from miscellaneous parts. These samples were held frozen (−17°C) until needed for analysis, then partially thawed, ground through a 0.63 cm die and subjected to proximate analysis.

Laboratory analysis

Proximate analysis. Proximate analysis (moisture, protein, fat and ash) was performed on all fractions collected in the laboratory and pilot scale extractions, and on the MSCR samples collected to assess the degree of variability. Moisture assay was conducted on 25-g duplicate samples dried in a forced air oven at 95°C to constant weight. Nitrogen was assayed via the automated Kjeldahl method using the Kjel-Foss Automatic Model 16210 (Foss America, Inc., Fishkill, NY) (AOAC method 7.021, 1980) and protein calculated by multiplying by 6.25. Fat content was assayed by the Butt tube method (AOCS Official Method Aa-4-38, 1983). Ash was determined by a stepwise charring of the samples at 200°C for 2 hr, 300°C for 2 hr, and 600°C for 18 hr or until constant weight was achieved.

Microbiological analyses. Aerobic plate counts (APC; AACC method 42-11, 1983) were conducted for the MSCR starting material, supernatant 1 and precipitate 2 in the laboratory scale experiments on the effect of extraction temperature, and also for all pilot scale extractions on the starting materials (MSCR) and the final products (AECP). For each pilot scale run, the starting materials and final products (AECP). For each pilot scale run, the starting materials and final products were also tested for psychrophile count (AACC method 42-11 using Nutrient agar, spread plates, incubation at 7°C for 10 days), presumptive *Salmonella* (AACC method 42-25A to step 6), and presumptive coliform-*E.coli* (AACC method 42-15 to step 5).

Statistical analysis. Where appropriate, data were subjected to one way analysis of variance and treatment means compared using Duncan's New Multiple Range test (Steel and Torrie, 1960).

RESULTS & DISCUSSION

Laboratory experiments

The results of statistical analyses of the data from the laboratory experiments to test the effects of grinding, temperature and centrifugal force on the protein extraction and recovery process are summarized in Table 1. For most of the fractionated materials, the effects of altering grinding, temperature or centrifuge force were not significant. For those that were significant, the data are presented in Tables 2–4 and are discussed below.

Table 5—Aerobic plate count for selected steps in the laboratory scale preparation of protein from mechanically separated chicken residues at three temperatures

Fraction	10°C Rep 1	10°C Rep 2	22°C	32°C Rep 1	32°C Rep 2	32°C Rep 3
			APC/g			
Starting material	6.9×10^7	3.3×10^7	9.6×10^6	5.0×10^7	8.5×10^8	2.2×10^7
Supernatant 1	2.3×10^6	2.3×10^6	2.5×10^6	8.0×10^5	6.6×10^7	5.0×10^5
Precipitate 2	1.9×10^6	2.0×10^5	4.0×10^5	1.5×10^6	1.9×10^8	1.7×10^6

Table 6—Fractionation of material and composition of fractions (as-is basis) obtained from pilot scale recovery of protein from mechanically separated chicken residues while varying grind, temperature, and centrifuge type[a]

	Unground (A)	Ground (procedures B-F)				
	Ambient temperature (procedures A-D)				Cold temperature (E,F)	
	Basket centrifuge (A,B)		Decanter centrifuge (C,D,E)			Basket centrifuge (F)
Fraction and assay	A	B	C	D	E	F
Starting material	(n = 3)[c]	(n = 1)	(n = 2)	-[d]	(n = 1)	-[g]
Moisture, %	61.2	62.0	58.2	-[d]	62.8	-[g]
Protein, %	17.9	18.1	17.4	-[d]	17.2	-[g]
Fat, %	5.8	6.3	14.1	-[d]	6.4	-[g]
Ash, %	9.9	8.5	8.6	-[d]	8.5	-[g]
Solids 1 (Bone residue)	(n = 3)	(n = 1)	(n = 3)	(n = 1)	(n = 1)	(n = 1)
Percentage wet wt[b]	46	28	49	44	72	40
Percentage dry matter wt[b]	77	79	58	49	81	92
Percentage protein wt[b]	87	59	72	58	88	87
Moisture, %	70.9	52.4	78.2	78.3	80.6	60.3
Protein, %	14.9	16.7	11.7	12.0	9.6	17.2
Fat, %	2.7	7.4	1.1	1.2	1.7	6.0
Ash, %	9.2	16.9	7.8	7.1	6.2	16.8
Effluent 1 (solubilized protein)	(n = 1)	(n = 2)	(n = 3)	(n = 1)	(n = 1)	(n = 2)
Percentage wet wt	51	59	47	57	24	44
Percentage dry matter wt	29	16	38	30	9	14
Percentage protein wt	24	20	21	19	17	16
Moisture, %	90.3	95.3	85.2	89.0	94.4	94.7
Protein, %	3.7	2.7	3.6	3.2	2.8	3.0
Fat, %	2.9	0.8	9.9	4.1	1.4[f]	1.0
Ash, %	0.7	0.5	0.6	0.6	0.5	0.5
Solids 2 (Protein sludge)	(n = 1)	(n = 1)	(n = 3)	(n = 1)	(n = 2)	(n = 1)
Percentage wet wt	19	15	10	17	10	14
Percentage dry matter wt	11	10	11	16	8	9
Percentage protein wt	13	13	10	16	9	10
Moisture, %	89.5	88.4	81.1	81.0	87.5	89.4
Protein, %	5.5	6.6	7.5	8.8	6.8	6.1
Fat, %	2.4	2.2	9.6	5.3	2.2[f]	1.9
Ash, %	0.5	0.5	0.6	0.5	1.0	0.5
Effluent 2	(n = 1)	(n = 1)	(n = 2)	(n = 1)	(n = 1)	
Percentage wet wt	36	40	28	34	13	28
Percentage dry matter wt	4	5	18	14	4	-[h]
Percentage protein wt	2	6	6	4	4	-[h]
Moisture, %	98.3	97.8	87.7	91.6	94.5	-[h]
Protein, %	0.5	1.0	1.6	1.1	2.6	-[h]
Fat, %	0.7	0.3	9.9	3.5	0.7	-[h]
Ash, %	0.2	0.6	0.5	-[e]	1.6	-[h]

[a] Procedure C was conducted November 3, 1983; procedures B,D,E, and F on February 1, 1984; and procedure A on March 7, 1984.
[b] Percentage wet weight, percentage dry matter weight and percentage protein weight refer to the percentage of the total amount of that component originally present in the MSCR slurry, which was measured in the fraction.
[c] The n = number of samples taken and assayed in duplicate to obtain values.
[d] Same starting material as for procedure B.
[e] Sample not available.
[f] Fat skimmed from effluent 1.
[g] Same starting material as for procedure E.
[h] No sample of effluent 2 was taken.

Grinding experiments. Generally, the main effect of grinding the starting material was to decrease the percent of dry matter in precipitate 1 with a resulting increase in the percentage of dry matter in supernatant 1 (Table 2). A higher percentage of the original protein was also noted in supernatant 1 when the starting material was ground, but this was not reflected in precipitate 2 (the final product). The ash content of the final product was higher when the MSCR was ground. There was little difference between grinding through a 0.63 cm or 0.32 cm die.

In the grinding experiments, the fat content of the starting materials was not uniform among treatments (Table 2). This may have been due to inadequate mixing of the 16 kg batch of starting material used for all laboratory experiments, or to inadequate sampling, although careful precautions had been taken. The higher fat content of the unground starting material must be considered as an explanation for the higher fat content of the resulting precipitate 2, in comparison to the starting material ground through a 0.63 or 0.32 cm die.

Temperature experiments. Varying extraction temperature produced the greatest number of significant effects on the fractionation of materials and on fraction composition. The fat content of the fractions was consistently affected by extraction temperature (Table 3), but the results are difficult to explain.

Table 7—Microbiological test results for pilot scale protein extractions of MSCR[a]

Sample[b]	Aerobic plate count (APC/g)	Psychrophile count (organisms/g)	Presumptive Salmonella assessment (organisms/g)	Presumptive coliform count (organisms/g)
Procedure C Ground/Ambient/DC (high fat)				
MSCR	1.2×10^{6}	6.8×10^{5}	No typical colonies observed.	1.0×10^{5}[c]
AECP	7.9×10^{5}	7.8×10^{4}	No typical colonies observed.	4.5×10^{5}[c]
Procedures B,D,E,F				
MSCR	9.9×10^{6}	–d	Typical colonies and positive test on TSI observed for all materials.	All tubes positive at all 3 dilutions. >2400 organisms/g by MPN for all materials.
AECP:				
Ground/Cold/DC	1.4×10^{7}			
Ground/Cold/BC	1.8×10^{6}			
Ground/Ambient/DC	TNTC (>3×10^{7})			
Ground/Ambient/BC	4.1×10^{6}			
Procedure A Unground/Ambient/BC				
MSCR	3.6×10^{5}	4.0×10^{5}	Typical colonies and positive test on TSI observed for both materials.	All tubes positive at all 3 dilutions. >2400 organisms/g by MPN for both materials.
AECP	1.1×10^{7}	1.1×10^{5}		

[a] Procedures A through F are the same as described in Table 6.
[b] DC = decanter centrifuge; BC = basket centrifuge.
[c] Sample blended with buffered water, appropriate dilutions made, plated onto MacConkeys Agar and typical colonies counted.
[d] Refrigerated incubator (7°C) failed and all plates overgrew (TNTC).

Table 8—Proximate composition of mechanically separated chicken residue samples collected over a 2-1/2 month period[a]

Type of feed to deboner		Moisture	Protein	Fat	Ash
		(%, as-is basis)			
Necks (n = 8)[b]	mean	61.6 ± 1.0a	18.0 ± 0.9	8.7 ± 1.9b	10.4 ± 1.5
	range	(60.5 – 63.2)	(17.3 – 19.6)	(6.0 – 10.9)	(8.5 – 13.0)
Backs (n = 10)	mean	58.0 ± 1.5b	18.2 ± 0.9	11.7 ± 2.9a	10.8 ± 2.4
	range	(56.1 – 61.0)	(16.3 – 19.6)	(6.8 – 16.1)	(6.3 – 13.9)
Necks & Backs (n = 13)	mean	59.1 ± 2.0b	18.7 ± 1.1	9.1 ± 2.1b	11.8 ± 2.1
	range	(56.7 – 62.6)	(16.7 – 20.3)	(5.7 – 12.6)	(8.2 – 14.7)

[a] Means within a column (composition parameter) followed by different letters are significantly different at the 5% level.
[b] The n = the number of samples of this type collected and analyzed.

Table 9—Typical composition of protein material prepared from mechanically separated chicken residue on the laboratory and pilot scales, compared with mechanically deboned poultry meat

Material	Moisture	Protein	Fat	Ash
	(%, as-is basis)			
Laboratory produced AECP (Precipitate 2)	80-88	8-10	1-4	0.4 - 0.7
Pilot scale produced AECP (Solids 2)	81-89	6-9	2-10	0.4 - 0.6
Mechanically deboned poultry meat (MDPM)[a]	62-70	12-17	14-23	0.9 - 1.8

[a] Hamm and Young, 1983.

More fat was found in supernatant 1 (and less in precipitate 1) when the extraction was done at 22°C, as compared to 10° or 32°C. During protein precipitation, the fat present in supernatant 1 was deposited to a lesser degree in the final product (precipitate 2) with increasing temperature (data not shown, but calculated from dry matter weights and percent fat in Table 3). The difficulty in using a consistent technique in skimming fat accounts for the high standard deviations observed. The percentage of original protein and dry matter recovered in the final product (precipitate 2) was significantly greater at 22°C than at 10° or 32°C. Duerr and Earle (1974) have noted that the solubility of non-collagen protein increased with increasing extraction temperature from 60° to 80°C, but information on extractions at ambient or lower temperatures has not been found.

Centrifugal force experiments. Increased centrifugal force (14,000 × *g* as compared to lower values) produced smaller (lower percentage of wet weight), dryer precipitates (Table 4). The quantities of dry matter and of protein recovered in the final product were not affected by varying the g-force. Interestingly, increasing the centrifugal force decreased the quantity of ash in the final product (precipitate 2).

Microbiology. MSCR, which had been stored frozen after receipt from the poultry processing plant, contained between 9.6×10^{6} and 8.5×10^{8} APC/g (Table 5). This is grossly higher than the standard plate count bacterial levels of 1.5×10^{3} CFU/g for residues of mechanical deboning operations, or even of abused residue (1.7×10^{4} CFU/g after 5 hr at 23°C), observed by Jackson et al. (1982).

The counts decreased between 4- and 60-fold in supernatant 1, a larger decrease than would be expected by dilution alone. Thus, a high proportion of organisms remained in precipitate 1, a desirable situation since precipitate 1 is discarded. The counts decreased slightly in precipitate 2 in comparison with supernatant 1 for the 10° and 22°C extraction temperature, but consistently showed a slight increase for the 32°C extraction temperature. Compared to the starting material, organisms in the final product were reduced 4- to 150-fold, which is similar to the observations of Jackson et al. (1982).

Pilot scale extraction

Pilot scale extraction and recovery of protein from MSCR was conducted on four separate dates. The first attempt was terminated before any meaningful data could be gathered because the unground MSCR caused blockage of the lines and

of the decanter centrifuge. Blockage of this type had occurred previously (Lawrence et al., 1982), but the MSCR had been obtained from a processing plant using a Sepramatic deboner. It had been thought that the residue used in these experiments, which had been ground through a 1.9 cm die prior to feeding to the Beehive deboner, would function satisfactorily in the process.

The remaining three run days incorporated variations of grinding, extraction temperature, and centrifuge type, and the results are presented in Table 6. The composition of the starting material did not vary much except that the fat content of the starting material for procedure C was about twice as high as for the other procedures. This observation prompted a study of MSCR composition, which is discussed later. The pilot scale data in Table 6 are more variable than those collected in the laboratory experiments because of difficulties in collecting representative samples, obtaining exact fraction weights, and holding fixed parameters constant among pilot scale runs. Unfortunately, the cost to replicate pilot scale runs is very high, so that elimination of these difficulties was not always possible.

A number of occurrences during the pilot scale run were not anticipated by the laboratory trials. Grinding the MSCR through a 0.32 cm die was necessary to prevent blockage of the 2.5 cm lines and equipment used; a 0.63 cm grind appeared to be insufficient. The temperature of the MSCR rose from about 4° to 35°C during grinding. During the centrifugal separation of the solubilized protein from bone residue, the double flight scrolling in the decanter centrifuge plugged, while the single flight configuration performed successfully. In several runs, an attempt was made to manually skim fat from the surface of effluent 1 to reduce the fat content in the final products. This was only successful for the cold temperature run using the decanter centrifuge. At ambient temperatures, the decanter centrifuge appeared to emulsify the fat in the protein solution so that very little could be recovered by skimming the surface. In the basket centrifuge, the bone residue material depositing against the cloth-lined, perforated walls of the basket acted as an effective barrier for trapping fat, thus preventing its passage into the effluent.

The results of the various treatments tested at the pilot scale are discussed below.

Grinding vs Nongrinding. There were no major differences between procedures A and B (Table 6), which were conducted at ambient temperature using the basket centrifuge to separate solubilized protein from bone residue and which allowed comparison of grinding with not grinding prior to extraction. The composition of the AECP recovered was quite similar. It had been hypothesized that during grinding the fat and protein might form an emulsion leading to higher fat extraction and higher fat content in the AECP. This was not the case, at least when a basket centrifuge was used. Of the protein originally present in the MSCR, 13% was recovered in each case. The results do not parallel those observed in the laboratory experiments on effects of grinding (Table 2).

Temperature effects. The effects of extraction temperature can be assessed by comparing procedures B and D (18–22°C) with procedures F and E (3 – 7°C) (procedure C was not included due to the high fat starting material) (Table 6). Higher percentages of the original wet weight, dry weight and protein weight appear in solids 1 (bone residue) and consequently lower percentages in the solubilized protein effluent 1, when cold extraction temperatures were used. Similarly in the AECP (solids 2), recovery of materials was higher at ambient temperature. This is contrary to observations by Jelen et al. (1978), who noted a marginal improvement in protein yield during alkaline extraction of beef bones at 7–10°C. However, there is general agreement with the laboratory scale results in this study. Fat recovery in the AECP was higher at ambient temperature than at cold temperature for the decanter centrifuge process. When the decanter centrifuge process was used at the lower temperature, fat was skimmed from the surface of effluent 1. The cake which built up in the basket centrifuge apparently acted as an effective filter for fat regardless of temperature.

Centrifuge effects. The effects of using a decanter centrifuge or a basket centrifuge were determined by comparing procedures B and F with procedures D and E (Table 6). The basket centrifuge produced a smaller quantity of wet solids 1 (bone residue), that was substantially lower in moisture content. The basket centrifuge extracted a higher percentage of dry matter into solids 1. The basket centrifuge also concentrated more of the fat into the solids 1 (38 and 81 vs 14 and 42% of the original fat, calculated from data in Table 6).

Starting materials composition effects. When a high fat starting material was processed at ambient temperature through a decanter centrifuge (procedure C), the final protein product was substantially higher in fat than when a lower fat starting material was processed in the same manner (procedure D).

Microbiology. For aerobic plate counts, the final product contained approximately the same level of APC/g as the starting material, in spite of a dilution step (except for procedure A) (Table 7). The procedures conducted at ambient temperature produced higher counts in AECP than the corresponding procedure at cold extraction temperature. The basket centrifuge process produced lower counts than the extractor-centrifuge process, perhaps due to the filtering action.

Psychrophile counts were lower in the final product than in the starting material; unfortunately the psychrophile counts in the procedures comparing extraction temperatures and centrifuge type were lost due to incubator failure. The presumptive tests for *Salmonella* and coliforms indicated substantial contamination. This is not consistent with the observations of Jackson et al. (1982) in a more extensive study of the microbiology of the process at the laboratory scale and therefore requires further investigation.

Starting material composition

The results of the proximate analysis of 31 samples of MSCR are shown in Table 8. Using necks as feedstock to the deboner produced MSCR higher in moisture content than backs or necks and backs, while backs alone produced MSCR higher in fat. The protein content of MSCR was relatively constant, regardless of feedstock type. The ash content of MSCR varied considerably, but the variation was not consistent for material type. The MSCR from necks alone was less variable in composition than MSCR from the other materials, as indicated by the ranges shown, but may be because fewer samples of MSCR from necks were taken. The four lots of material used in the pilot scale extractions (Table 6) were nearly as variable in composition as the materials analyzed in this study of MSCR composition.

Product composition

The feasibility of recovering protein from mechanically separated chicken residue depends on the value of and market for the AECP product and the cost of recovering it. The typical, as-is composition of AECP produced on both the laboratory and pilot scale in this study is compared (Table 9) with the composition of mechanically deboned poultry meat (MDPM). AECP was substantially higher in moisture and lower in fat than MDPM. On an as-is basis, AECP was somewhat lower in protein and ash contents than MDPM. Recalculated data from work by Golan and Jelen (1979) indicates the collagen content of AECP to be about 8 percent, using the conversion factors of Duerr and Earle (1974). As indicated in the introduction, other investigators have indicated that the use of AECP in sausage products appears promising (Caldironi and Ockerman, 1982; Jelen et al., 1982).

The yields of total protein obtained in the pilot scale runs varied from 9 to 16 percent of the original protein present in

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A Comparison of Mechanically Separated and Alkali Extracted Chicken Protein for Functional and Nutritional Properties

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ABSTRACT

Studies were carried out to determine functionality and nutritive value of alkali extracted, acid precipitated protein (EP) from bone residues of mechanically separated poultry. The evaluations were in comparison to mechanically separated chicken protein paste (PP), both fresh and after freezing and thawing. Measurements of the functional properties included the nitrogen solubility index (NSI), water absorption capacity (WAC), emulsifying capacity (EC), emulsion stability (ES) and heat gel strength (HGS). The NSI, EC and HGS values for the fresh and frozen PP paste were significantly higher ($P < 0.05$) than those for the respective samples of EP. Either fresh or frozen EP stabilized a test emulsion better than the PP. These differences might have been caused by the uneven moisture and fat contents of the two test materials. Nutritional quality of both products determined by the body weight gain, net protein utilization, protein efficiency ratio, and true digestibility tests was high and equal to or better than casein.

INTRODUCTION

THE MECHANICAL SEPARATION ("deboning") technology has been accepted by the poultry and pork processing industries. While the mechanically separated protein paste has found commercial applications as a valuable ingredient in various comminuted meat products, the ground poultry bone residue still contains 13–15% protein, 16–20% fat, and 8–11% ash (Lawrence et al., 1982). Aqueous processes for meat protein extraction from mechanically separated poultry residues (MSPR) and other meat-containing waste materials were proposed using NaCl (Young, 1975; Kijowski and Niewiarowicz, 1985) or dilute alkali (Jelen et al., 1979; Lawrence et al., 1982) and use of the extracted protein in luncheon meats and sausages investigated (Jelen et al., 1982; Kijowski et al., 1985).

When alkali extraction is used in the preparation of various protein concentrates, some damage of amino acids may occur, resulting in a reduction of protein quality (Aymard et al., 1978; Cheftel, 1977; Feeney, 1977; Friedman, 1977). Although no measurable lysino-alanine was induced by the alkali-extraction, HCl-precipitation process (Lawrence and Jelen, 1982b), concern might be expressed about the nutritive value of the alkali-extracted chicken protein material.

The manufacture of comminuted meat products is dependent on the functionality of the protein used in these products. The functional properties of protein may be divided into three groups which are characterized by molecular interaction of protein and water, protein, water and fat, and protein, water and air. The molecular interaction determines such properties as protein solubility, water absorption capacity, water binding ability, viscosity, gelation and swelling ability, emulsifying stability, or foaming powers (Hermansson, 1973).

In addition to the factors related to the protein itself (kind of material, method of preparation), the functional properties of protein depend on environmental factors including the presence of non-protein components (especially salt and its concentration), pH or temperature (Ozimek et al., 1981; Hermansson and Akesson, 1975). Many of these factors are related to the intended use of the protein in a specific food system. Thus, an evaluation of protein functionality may give results that could be applicable only in certain industrial situations.

The objective of the present study was to evaluate nutritive value and functionality of alkali extracted, acid precipitated protein (EP) from mechanically separated poultry residues. The evaluations were carried out in comparison to mechanically separated chicken protein paste (PP). Both products were evaluated fresh and after freezing and thawing, using various environmental conditions related to possible uses of the extracted protein in comminuted meat products.

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MATERIALS & METHODS

Preparation of alkali-extracted protein from poultry residues

Mechanically separated poultry residues (MSPR) were obtained from an industrial mechanical deboning operation using a Beehive AUX5 2445 deboner (Beehive Machinery Inc., Sandy, UT). The raw material for the mechanical separation process was from unskinned chicken backs, necks and spent layers. The MSPR were subjected to the alkali-acid extraction process as described by Lawrence et al. (1982) and Jelen et al. (1979) (20% NaOH at final pH 10.5, temperature 23°C and exposure time 30 min with subsequent acid precipitation at pH 5.4 by 3N HCl). Details of the pilot-scale process used are given elsewhere (McCurdy et al., 1986).

Extraction was carried out at the POS Pilot Plant Corporation in Saskatoon. The samples were shipped by plane on the day of extraction and delivered to the Dept. of Food Science, Univ. of Alberta, for the functional property and nutritional studies. Industrially produced, mechanically separated (deboned) chicken ("protein paste" - PP), used as a reference (Table 1), was obtained from the same plant and the same feedstock.

One portion of each of the products was stored in a refrigerator (4°C) until all of the analyses on the fresh product were completed. The second portion of the fresh products was frozen at −20°C in plastic containers with well fitting lids and kept no longer than three months. Before experimental use, these samples were taken from the freezer and placed in a 2–4°C refrigerator for 15 hr to complete thawing.

Chemical analyses

Nitrogen, fat, moisture and ash were determined according to AOAC (1975). Amino acid analyses were performed on a Beckman 121 MB amino acid analyser with model 126 data system using the method outlined by Sarwar and Bowland (1975). Tryptophan and cystine were not determined.

Functional properties

Considering that the average concentration of sodium chloride in comminuted processed meat products is about 2% (Hamm, 1973), it was decided to carry out certain functional property tests without and with the addition of 2% NaCl.

The soluble protein content as Nitrogen Solubility Index (NSI). The NSI was determined by the method of Inklaar and Fortuin (1969) with two modifications. The extraction procedure was carried out at room temperature (20–21°C) and the Lowry method was applied to determine soluble protein (Lowry et al., 1951). Bovine serum albumin was used to provide the calibration curve for the soluble protein. The

Table 1—Chemical composition and pH of the fresh (A) and freeze-dried (B) mechanically separated protein paste and alkaline extracted protein

Product	Water (%)		Protein (%)		Fat (%)		Ash (%)		pH[a]
	A	B	A	B	A	B	A	B	A
Paste	66.84	3.65	9.94	28.96	22.56	65.55	0.63	1.85	6.62
Extracted protein	88.46	2.53	6.08	50.06	5.05	42.63	0.44	3.72	5.40

[a] Active acidity of the product was determined using the Beckman Model 4500 digital pH meter. Ten grams of sample were macerated with 10 cm^3 of distilled water, the pH being measured 0.5 hr after maceration.

NSI test was determined for NaCl concentrations of 0, 0.3, 0.5, 1.0, 1.5, and 2.0M. The results were calculated as percent soluble protein in the total protein.

Water absorption capacity (WAC). A modification of the method developed by Jay (1964) was utilized to measure the water absorption capacity of the protein in the various samples. This test was performed with 15-g samples over a temperature range 20–85°C using dispersions of the material in either distilled water or 2% NaCl solution. The appropriate liquid was added in an amount such that the ratio of protein to added liquid was 1:5 (w/w), regardless of the initial water content of the samples.

An exact amount of product was weighed into an 80 mL beaker, the respective liquid was added, and the contents were mixed with a stainless steel spatula. The beakers were covered and immersed in a water bath set to the test temperature. Preliminary investigations had established the appropriate immersion times required for the test materials to reach the various desired temperatures. After the correct heat treatment had been applied, the sample was transferred to a filter funnel, lined with No. 1 Whatman filter paper and covered to minimize evaporative losses. The volume of solution released into a graduated cylinder was recorded.

The WAC (g of water absorbed per g of protein) was calculated as

$$WAC = \frac{A\text{-}B}{C}$$

where A - amount of water or 2% NaCl solution added to the prepared sample, g; B - amount of liquid released during test, g; C - amount of protein in the sample, g. All measurements were in triplicate.

Emulsifying capacity (EC). The capacity of the test materials to form an emulsion with oil was measured using a method developed by Swift et al. (1961) and modified by Kwasniewska et al. (1976). This method measures emulsifying capacity by utilising the fact that a large change in electrical resistance occurs when an emulsion inverts. The set for serial routine measurements consisted of a "Sorvall Omni-mixer" with regulated speed, a 250 mL glass beaker, and an attachment of copper electrodes 95 mm long and 2 mm in diameter. The electrodes were connected to a resistance-meter. Canola oil of commercial quality was titrated into the test material by a glass tube, connected to a graduated vessel.

An exact amount (20 mL) of a protein dispersion containing 50 mg of protein was placed in the glass beaker, the electrodes with a resistance-meter were attached, and the dispersion was pre-blended for 60 sec at a given mixer speed. Canola oil from the graduated vessel was then introduced at a constant flow-rate until a substantial increase in the resistance of the test system was observed.

The emulsifying capacity was expressed as V_{100} (mL of oil emulsified by 100 mg of protein):

$$V_{100} = \frac{100 \times b}{a}$$

where a - amount of protein in the sample, mg; and b - volume of oil required to invert the emulsion, mL.

Preliminary investigations had established the following optimal measurement conditions of EC that gave the most reproducible results: oil-flow rate, 0.35 mL/sec; mixing speed, 2000 rpm; amount of water-protein phase, 20 mL; protein content in the water-protein phase, 50 mg. All measurements were performed at room temperature. Dispersions of the products in either distilled water or 2% NaCl solution were tested.

For the determination of EC, the samples were prepared as follows: an exact amount of product that yielded a protein concentration of 50 mg in 20 mL of water-protein phase was weighed into a 250 mL beaker and an exact amount of respective liquid was added to make up to 150 mL in total. The system was mixed with a stainless steel spatula, stirred on the magnetic stirrer for 15 min, and kept in the refrigerator (5 ± 1°C) for 20 hr. Before the test was performed, the samples were warmed up to the 20–21°C room temperature. Averages of six determinations were calculated.

Emulsion stability (ES). Emulsion stability characteristics were evaluated as described by Inklaar and Fortuin (1969). In this procedure, 2.5-g samples were dispersed in 45 mL distilled water at 20°C for 15 min, 1.5g NaCl was then added and stirring was applied for 1 min. Next, 25g of soybean oil was slowly added to the dispersed protein while stirring at 1,000 rpm. When all of the oil was added, the stirring was continued for additional 60 sec. The content of the beaker was transferred into a 150 mL graduated centrifuge tube, which was placed into a water bath at 85°C for 15 min. The tube was then cooled under running tap water for 15 min and centrifuged for 15 min at approx. 800 × *g*. The amount of oil separated was read off and the centrifuging was repeated until the volume of separated oil did not change any further. The separated oil was calculated as a percentage of the total amount of the oil added. Four determinations were carried out to obtain the average emulsion stability data.

Heat gel strength (HGS). The method used to measure the heat gel strength was based on the test described by Mitchell (1976). This measurement was performed with 1:5 w/w protein to added liquid dispersions of the test materials in either distilled water or 2% NaCl solution as with the WAC. Samples of the products alone without any water or 2% NaCl solution added were also tested.

Equal samples (30g) of the products alone or the aqueous dispersions of the products were thoroughly mixed with a spatula in 80 mL beakers. The beakers were covered and immersed in a boiling water bath, where they were heated until the internal temperature of the samples reached the selected temperature (70°C or 85°C). The heated samples were cooled in an ice bath to internal temperature of 5°C and held at this temperature overnight.

On the following day the samples were tested on an Instron universal testing machine (Instron Corp., Canton, MA). A 3.6 cm diameter stainless steel flat probe was attached to the drive shaft of the Instron (Model 1132 Food Testing System) which had been set to a cross head speed of 10 cm/min with a chart speed to cross head speed ratio of 2:1. Each beaker was centered on a platform under the driveshaft, and the probe was driven into the sample, compressing it to 30% of its original height. The heat gel strength was recorded as the maximum force (kg) required to compress or penetrate the gel as read off the force deformation curve. One reading per beaker was taken and visual observations of the gel characteristics were also recorded. Averages of five determinations with 5 different beakers were calculated for each type of material.

Nutritional evaluation of protein quality

For nutritional studies, the fresh products were freeze-dried and stored in sealed plastic bags in the refrigerator until they were used to formulate the diets for the rats (Table 2).

Protein efficiency ratio (PER). The standard method (AOAC, 1975 - method no. 43.183-187) was followed. Thirty weanling male and female rats (Sprague-Dawley), 21–28 days of age with an average body weight of 58.5g were divided into three groups with weight variations as small as possible. Each group of 10 animals was randomly assigned to the experimental diets which were calculated to be isocaloric, isofibrous and isonitrogenous. The rats were individually housed in metabolic cages in an air-conditioned room at 25°C and provided with water and test diet *ad libitum*. Body weight and food consumption data were collected at weekly intervals through the 28-day test period. The ratio of weight gain to protein consumption was calculated for each rat and the average group values were statistically treated. The PER values obtained were corrected by a factor such that the value for the casein standard was 2.50.

Net protein utilization (NPU). Two sets of four weanling rats (2 male and 2 female in set), 26-28 days of age, with an initial weight range of 54.15 ± 2.52g were used in each group for the NPU experiment, carried out according to Miller and Bender (1955). The rats were individually housed in metabolic cages and provided with water and test diet *ad libitum*. Additionally, one batch of rats on a nonprotein

Table 2—Formulation of the experimental rations

Ingredients	Casein	Paste	Extracted protein	Protein free diet
	Source of protein			
Casein[a]	11.50	—	—	—
Paste	—	34.56	—	—
Extracted protein	—	—	19.98	—
Canola oil	22.65	—	14.12	22.65
Mineral mixture[b]	5.00	4.37	4.26	5.00
Vitamin mixture[b]	1.00	1.00	1.00	1.00
Alphafloc	1.00	1.00	1.00	1.00
Water	5.00	3.74	4.49	5.00
Cornstarch	53.85	55.33	55.15	65.35
Crude protein (total N × 6.38 for the casein and total N × 6.25 for the paste and extracted protein)	11.44	10.56	10.57	0.74

[a] NRC reference casein.
[b] AOAC (1975).

diet was used. The experiment lasted 10 days. Prior to the conclusion of the experiment, the rats were starved for 18 hr, weighed, sacrificed with chloroform and dried at 105°C for 48 hr. Carcass N was calculated according to the method of Rafalski and Nogal (1964).

Protein digestibility. Total feces were collected from each rat during the last five days of the NPU experiment. Feces were freeze-dried and ground in a laboratory mill through a 0.8 mm mesh. The values of true digestibility (TD) were calculated according to the following formula:

$$TD = \frac{\text{Total N intake - (Faecal N - Metabolic N)}}{\text{Total N intake}} \times 100$$

Metabolic N values were obtained from the batch of rats on the nonprotein diet.

Statistical analyses

In all functionality and nutritional value experiments, significant differences among treatment means were established by the Student-Newman-Keuls and Duncan multiple range tests. The University of Alberta computer system with its standard library of statistical programs was used in all statistical evaluations.

RESULTS & DISCUSSION

Functional characterization of test products

The Nitrogen Solubility Index (NSI). The values of NSI for the fresh and frozen chicken protein paste (PP) were significantly higher ($P < 0.05$) than those for the respective extracted protein (EP) samples (Table 3). For the PP the freezing process resulted in a decrease of the NSI, while for the extracted protein an opposite tendency was found. The NaCl content in the extraction medium had little effect on soluble protein in fresh as well as frozen EP, whereas for the paste, the protein solubility index was salt concentration dependent and increased substantially with the increase of NaCl content from 0 to 1M in the samples (Table 3). The NaCl content of the EP samples was likely somewhat higher than that of the PP, due to the nature of the extraction-precipitation processes used; whether this slight difference contributed to the effects observed could not be determined.

Solubility is an important property governing the functional behavior of proteins and their potential use in food processing. Generally, soluble proteins possess other functional attributes (e.g., emulsification) considered superior for most food processing applications. However, solubility and emulsifying properties may not be closely correlated as indicated by several contradictory reports (Aoki et al., 1981; Smith et al., 1973; Wang and Kinsella, 1976).

Freezing may have caused some denaturation of protein in the PP which contributed to the observed loss of solubility (Table 3). Saffle and Galbreath (1964) suggested that frozen meat is 8% less effective than fresh meat in certain functionality aspects. On the other hand, the freezing process resulted in an apparent increase of the content of soluble protein in EP (Table 3). As shown by Torgersen and Toledo (1977), both freezing and frozen storage may cause changes in myofibrillar protein of poultry meat which may have affected the functional properties of these products.

Water absorption capacity (WAC)

The differences in WAC between the paste and the extracted protein for all fresh and frozen samples (Fig. 1 and 2) were significant ($P < 0.05$). The water absorption capacity values for the fresh and frozen EP were all negative, which means that under conditions of the test, the product was losing its "own" water, the amount of lost water being higher for the frozen samples. The presence of NaCl ions slightly reduced the amount of water lost by the fresh and frozen extracted protein and substantially improved the WAC of the fresh and frozen paste (Fig. 2). Regardless of the product, for most of the samples the WAC decreased with the increase of temperature. These results correlate with the observation made by Torgersen and Toledo (1977) who reported that the WAC of the low temperature extracted chicken protein continuously dropped in the range of temperature from 4.5°C to 78.5° and drastically between 78.5 and 90°C. On the other hand, chicken protein showed gradual increases in solubility with increases in temperature (Torgersen and Toledo, 1977). The higher negative values for WAC for all fresh and frozen alkali-extracted protein from poultry chicken might have been due to differences in the kind of protein contained in the PP or EP; changes in protein structure, denaturation, solubility, hydrophobicity, hydrophile-lipophile balance and other factors caused by alkali-extraction followed by acid precipitation and temperature treatment (heating or freezing); or the differences in the moisture and fat contents of the EP and PP materials.

Emulsifying capacity (EC) and emulsion stability (ES)

The emulsifying characteristics of proteins were determined by emulsifying capacity and emulsion stability measurements. All differences in emulsifying capacity of our tested products were significant ($P < 0.05$) except for the frozen-thawed EP and PP when compared in water (Table 4). The freezing process

Table 3—Nitrogen solubility index[f] (NSI) of tested products, expressed as a percent of total protein

Sample	Paste: Fresh	Paste: Frozen	Sample	Extracted protein: Fresh	Extracted protein: Frozen
PP[g]-H_2O[h]	18.3 ± 0.1^{Aa}	13.3 ± 1.1^{Ba}	EP-H_2O	11.1 ± 0.0^{Ca}	13.2 ± 0.1^{Ba}
PP-NaCl (0.3 M)	22.1 ± 2.7^{Aa}	23.7 ± 0.0^{Ab}	EP-NaCl (0.3 M)	13.2 ± 0.7^{Bb}	21.3 ± 0.3^{Ad}
PP-NaCl (0.5 M)	36.1 ± 1.2^{Ab}	26.7 ± 1.5^{Bbc}	EP-NaCl (0.5 M)	12.8 ± 1.2^{Cb}	20.9 ± 0.6^{Dc}
PP-NaCl (1.0 M)	41.2 ± 1.8^{Ab}	30.2 ± 1.1^{Bcd}	EP-NaCl (1.0 M)	13.2 ± 0.4^{Cb}	21.4 ± 0.1^{Dcd}
PP-NaCl (1.5 M)	35.1 ± 2.7^{Ab}	32.4 ± 0.1^{Ade}	EP-NaCl (1.5 M)	13.0 ± 0.6^{Bb}	20.6 ± 0.0^{cbc}
PP-NaCl (2.0 M)	35.5 ± 0.9^{Ab}	33.9 ± 2.7^{Ae}	EP-NaCl (2.0 M)	12.7 ± 0.0^{Bab}	20.0 ± 0.3^{cb}

[A-D] Means with different superscripts in the same row differ from each other ($P < 0.05$).
[a-e] Means with different superscripts in the same column differ from each other ($P < 0.05$).
[f] Mean ± st. dev.
[g] PP (or EP) indicates the type of product (PP - protein paste, EP - extracted protein).
[h] H_2O (or NaCl) indicates the medium (either distilled water or 2% NaCl solution) in which the product was dispersed.

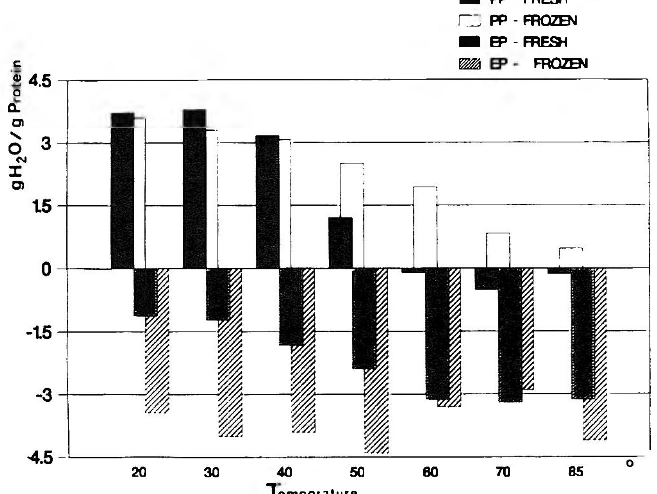

Fig. 1—Water absorption capacity of protein paste (PP) and extracted protein (EP). Medium - water. (Average standard deviation of all data 0.15, range of st. dev. 0.0-0.38).

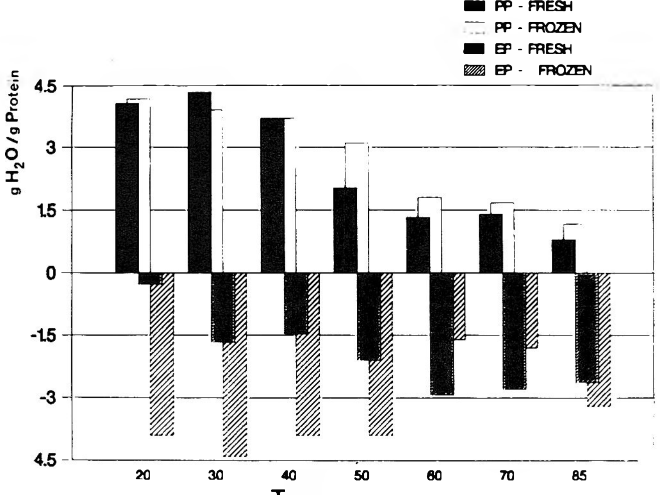

Fig. 2—Water absorption capacity of protein paste (PP) and extracted protein (EP). Medium - 2% NaCl in water. (Average standard deviation of all data 0.19, range of st. dev. 0.0-0.76.)

significantly ($P < 0.05$) decreased the emulsifying capacity in both products. The addition of NaCl ions improved the emulsifying capacity in all tests ($P < 0.05$). Although statistically significant, the differences between the PP and the EP were not large and may have been again due to the substantially higher amount of moisture in the EP relative to the amount of protein present.

There is a qualitative difference between the emulsifying capacity and the emulsion stability. Emulsion stability is not a measure of maximum oil addition, but rather of the ability of the product to retain its characteristics. The EP - either fresh or after freezing and thawing - stabilized the emulsion better ($P < 0.05$) than the PP (Table 5). This may have been related to the much lower fat to protein ratio in the EP (0.8) as compared to the PP (2.26). The freezing process caused a significant ($P < 0.05$) decrease in the ability of both products to stabilize the emulsion.

Heat gel strength (HGS)

The fresh and frozen PP formed heat gels that were significantly ($P < 0.05$) stronger than the gels formed by the fresh extracted protein (Table 6). The frozen extracted protein did not exhibit any gel-formation upon heating and cooling in the test conditions employed. After heating, the samples formed a clearly defined precipitate suspended in a free liquid. In contrast, the PP samples did not show any separation under the conditions of the test. It may be that the much higher moisture content of the EP samples, together with the relatively high fat content, influenced the gel-forming behavior. In addition, the mixing step in the sample preparation must have had a disruptive effect on the fibrous structure which is known to form upon freezing of the alkali extracted protein (Lawrence and Jelen, 1982a). Strength of all heat gels formed was affected by the salt and temperature conditions investigated, the temperature effect being more pronounced than the salt effect.

Nutritional evaluations

There were relatively large differences in the dry matter composition of the mechanically deboned chicken paste and the alkali extracted protein (Table 1). Significant differences ($P < 0.05$) were found also between the PP and EP in terms of the amino acid composition. The EP was characterized by higher ($P < 0.05$) content of isoleucine, leucine, phenylalanine, threonine and tyrosine (Table 7), while differences in lysine, histidine, valine and alanine were not significant.
The nutritional value of the protein in the two tested materials expressed by the NPU, PER and TD was high and not much different from that of casein (Table 8). There were no significant (at $P < 0.05$) differences between the NPU values of PP and EP; both were significantly ($P < 0.05$) higher than casein. The PER of the PP was the highest and significantly differed ($P < 0.05$) from that for the EP and the control casein. The TD indices of the paste and extracted protein were slightly lower than that of the casein; the difference between EP and casein was significant ($P < 0.05$).

Protein extracts should be of high nutritional value and nontoxic before they can be successfully accepted as food ingredients. The most commonly used method for preparing plant protein concentrates or isolates is alkaline extraction followed by precipitation of the extracted protein either by decreasing the pH to the isoelectric point or by heating. Exposure of protein to alkaline treatment, in addition to denaturation, may effect its nutritive value. Some of the undesirable side effects include racemization of AA, β-elimination and cross-linkage of AA and the formation of antinutritive substances (Cheftel, 1977; Feeney, 1977; Friedman, 1977).

In the present studies the nutritional quality of extracted proteins from MSPR was not substantially impaired by alkaline treatment and acid precipitation. Although protein efficiency ratio and body weight gain were significantly lower ($P < 0.05$) for EP when compared to PP (Table 8), the differences were small, and in both cases, the EP scored higher than the control casein. This indicates that the EP has a much higher nutritional value than the total bone residue protein (Wallace and Froning, 1979). Lawrence and Jelen (1982b) failed to demonstrate any

Table 4—Emulsifying Capacity[a] (EC) of tested products, ml oil/100 mg protein

	Paste			Extracted Protein	
Sample	Fresh	Frozen	Sample	Fresh	Frozen
PP-H_2O	165.2 ± 12.1	111.3 ± 7.1[b]	EP-H_2O	128.0 ± 4.0	112.0 ± 4.2[b]
PP-NaCl	181.3 ± 10.6	154.5 ± 4.4	EP-NaCl	161.8 ± 7.7	126.2 ± 9.8

[a] Mean ± st. dev.
[b] All differences except for frozen PP-H_2O vs frozen EP-H_2O were statistically significant ($P < 0.05$).

Table 5—Emulsion stability[d] (ES) of tested products, % of total amount of oil added

Paste		Extracted protein	
Fresh	Frozen	Fresh	Frozen
80.0 ± 3.3[a,A]	96.0 ± 3.3[b,B]	73.5 ± 3.3[a,C]	82.2 ± 1.3[c,D]

[a-c] The lower case letter indicates the effect of product (paste vs extracted protein) on ES.
[A-D] The capital letter indicates the effect of treatment (fresh vs frozen) on ES. Different letters indicate significant differences at $P < 0.05$.
[d] Mean ± st. dev.

Table 6—Heat gel strength[a] (HGS) of tested products, kg

Paste			Extracted Protein		
Sample	Fresh	Frozen	Sample	Fresh	Frozen
P-70	2.5 ± 0.3[b]	2.3 ± 0.2[b]	EP-70	0.3 ± 0.0	No gel formed
P-85	3.5 ± 0.3	5.5 ± 0.1	EP-85	1.3 ± 0.2	No gel formed
P-H_2O-70	2.9 ± 0.3	0.9 ± 0.1	EP-H_2O-70	0.1 ± 0.0	No gel formed
P-H_2O-85	4.0 ± 0.4	1.5 ± 0.1	EP-H_2O-85	0.3 ± 0.1	No gel formed
P-NaCl-70	2.3 ± 0.1	0.7 ± 0.0	EP-NaCl-70	0.1 ± 0.0	No gel formed
P-NaCl-85	3.1 ± 0.3	1.9 ± 0.3	EP-NaCl-85	0.2 ± 0.0	No gel formed

[a] Mean ± st. dev.
[b] All differences between fresh and frozen paste except P-70, and all differences between fresh paste and fresh extracted protein were significant at $P < 0.05$.

Table 7—Amino acid composition (g/16 g N) of test products[c]

Item	Paste	Extracted protein	Whole egg protein (Eggum, 1968)
Arginine	6.9[a]	6.2[b]	6.15
Histidine	2.6[a]	2.7[a]	2.54
Isoleucine	4.3[a]	4.5[b]	5.76
Leucine	7.6[a]	8.2[b]	8.90
Lysine	8.1[a]	7.8[a]	6.65
Methionine	2.7[a]	2.5[b]	3.01
Phenylalanine	3.7[a]	5.1[b]	6.69
Threonine	4.1[a]	4.3[b]	5.14
Tyrosine	3.3[a]	3.8[b]	3.63
Valine	4.6[a]	4.8[a]	7.54
Alanine	5.7[a]	5.3[a]	
Aspartic acid	9.3[a]	8.5[b]	
Glutamic acid	14.7[a]	13.4[b]	
Glycine	7.2[a]	4.2[b]	
Proline	5.4[a]	3.7[b]	
Serine	4.3[a]	3.9[b]	

[a,b] Different letters in the row indicate significant differences at $P < 0.05$.
[c] Recovery as a protein (N × 6.25) 90.8%. Tryptophan and cystine are not included in this analysis.

Table 8—Net protein utilization (NPU), protein efficiency ratio (PER), true digestibility (TD) and rat growth performance of test products

Source of protein	NPU[d]	NPU_c[e]	Body weight gain[d] $g \cdot rat^{-1}$ 28 d^{-1}	Feed intake[d] $g \cdot rat^{-1}$ .28 d^{-1}	PER[d]	PER_c[e]	TD[d] (%)
Paste	68.5 ± 1.3[a]	73.9	130.6 ± 4.1[a]	361.2 ± 8.2[a]	3.4 ± 0.1[a]	2.9	93.6 ± 0.2[a]
Extracted protein	69.1 ± 1.3[a]	74.5	110.8 ± 2.4[b]	336.1 ± 4.6[b]	3.1 ± 0.1[b]	2.6	93.2 ± 0.3[a]
Casein	64.9 ± 1.8[b]	70.0	102.9 ± 3.8[b]	301.2 ± 10.3[c]	3.0 ± 0.0[b]	2.5	94.2 ± 0.2[b]

[a,b,c] Data in each column followed by the same letter are not significantly ($P > 0.05$) different.
[d] Mean ± SEM.
[e] Values were corrected by using casein as a standard with PER = 2.5 and NPU = 70.

presence of lysino-alanine in the alkaline extracts suggesting that the extraction conditions are relatively nondestructive to the protein.

CONCLUSIONS

DIRECT COMPARISON of the functional properties data for the mechanically separated chicken paste and alkaline extracted chicken proteins might suggest that the paste exhibited better functionality than the extracted protein. Except for the ability to stabilize an emulsion, differences in all other functional properties (NSI, WAC, EC, HGS) were statistically significant at $P < 0.05$ and better for the paste. The functional properties examined in the present work were affected by the addition of salt, temperature conditions investigated and the freezing process; all of these had a statistically ($P < 0.05$) significant effect on the functional behavior of both products. The apparent differences in the functionality data for the EP and PP may have been due to the primary characteristics of the proteins contained in the PP and EP, and/or the compositional differences of the two products, particularly the much higher moisture content relative to the protein content, the higher fat content of the PP, and the additional NaCl produced by the extraction process. While most of the functionality tests were normalized as to the protein, the differences in moisture, fat and NaCl could have been important. As given, the results describe the actual performance characteristics of these two products obtained by currently available techniques. The alkaline extraction process appeared to have a slight effect on some indicators of nutritional quality (PER, body weight gain), while other tests (NPU, TD) showed little difference. In general, nutritive value of both tested products was high and equal to or better than that of casein.

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the MSCR; in the laboratory scale experiments, yields of from 6 to 15% were observed. Jelen et al. (1978) obtained a protein yield of 13 to 16% for beef bones.

Commercial processing of MSCR to produce food grade protein would require considerable care to avoid both microbial contamination and conditions for promotion of microbial growth, as the counts for AECP produced in these experiments are generally considered unacceptable (Wehr, 1982).

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N-Nitrosamine Ingestion from Consumer-Cooked Bacon

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ABSTRACT

Thirty-nine fried bacon samples and corresponding fried-out fats were collected from randomly selected consumers' homes. Bacons were purchased and cooked in the course of preparing meals containing bacon. The average volatile N-nitrosamine content (μg/kg) of the rasher and fried-out fat, respectively, were: N-nitrosodimethylamine, 4.0 and 5.3; N-nitrosopiperidine, 0.7 and 1.5; N-nitrosopyrrolidine, 17 and 32; N-nitrosothiazolidine, 8.9 and 3.4. The average daily ingestion of N-nitrosopyrrolidine from bacon for our survey respondents was estimated to be 76 ng/person/day. A majority of respondents indicated that they used the fried-out fat as a cooking oil.

INTRODUCTION

CONSIDERABLE RESEARCH effort has been directed at the occurrence of low μg/kg amounts of carcinogenic N-nitrosamines in human foods, especially fried bacon (Scanlan, 1983). At least three volatile (Hotchkiss and Vecchio, 1985) and one or more nonvolatile nitrosamines (Sen et al., 1985) have been detected in fried bacon and the fried-out fat.

These findings have prompted the Food Safety and Inspection Service (FSIS), U.S. Department of Agriculture, to promulgate regulations intended to lower or eliminate N-nitrosamines from fried bacon (Federal Register, 1978). These regulations set up a monitoring program to insure that the volatile N-nitrosamine content of the fried bacon was less than 17 μg/kg. Over 99% of the nearly 1000 samples collected from 241 plants in 1982 were in compliance (McCutcheon, 1984). Workers at the Food and Drug Administration found that the levels of volatile N-nitrosamines in fried bacon have decreased over a period of 10 years to average levels of <10 μg/kg (Havery and Fazio, 1985). The Committee on Nitrite and Alternative Curing Agents in Food (National Research Council, 1981) estimated that fried bacon contains 5 μg/kg volatile N-nitrosamines.

Several factors influence the N-nitrosamine content of cooked bacon including the amount of nitrite added (Sen et al., 1974), storage conditions and age of the meat (Pensabene et al., 1980), the fat to lean ratio (Fiddler et al., 1974), presence of nitrosation inhibitors in the cure mix (Gray et al., 1982), slice thickness (Theiler et al., 1981), smoking conditions (Mandagere et al., 1984), cooking method (Pensabene et al., 1974) and frying temperature and time (Hotchkiss and Vecchio, 1985).

Pensabene et al. (1974) found higher pan temperatures resulted in more NPYR even when the bacon was cooked to a similar doneness. The influence of temperature on NPYR formation has been investigated in model systems (Lee et al., 1983a). A clear relationship indicating that higher temperatures result in more NPYR was established. Theiler et al. (1981) showed that the relationship between NPYR content of the rasher and the thickness depended on frying time. Thicker slices formed less N-nitrosamine at 3 min/side but more at 5 min/side. We studied the effects of frying time on the total quantity of volatile N-nitrosamine (fried-out fat + rasher + vapor) formed during frying (Hotchkiss and Vecchio, 1985). The maximum amount of NPYR was consistently found after frying 3–4 min/side in a preheated (171° or 190°C) pan.

Most researchers and regulatory agencies have recognized that frying conditions strongly affect N-nitrosamine formation and have standardized frying conditions. FSIS specifies 340°F (171°C) for 3 min/side in its bacon monitoring program (Federal Register, 1978). These conditions are not likely to be those under which all consumers fry bacon. Lee et al. (1983b) pointed out that frying pan temperatures can vary considerably across their surface and heat settings may be inaccurate. Consumers are also likely to store bacon under conditions and for times that could influence nitrosamine formation. This means that nitrosamine levels determined under controlled laboratory conditions may not accurately reflect the levels in bacon being consumed.

N-nitrosamines in consumer purchased and cooked bacon have not been investigated. Wasserman et al. (1978) reported the N-nitrosamine content of two randomized lots of commercial bacon which were supplied by the authors and cooked at home by two groups of employees of the U.S. Department of Agriculture. The NPYR contents ranged from 3 to 14 and 1 to 8 μg/kg for the two lots, respectively. No correlation between nitrosamine content and cooking time or amount of heat applied, as estimated by the subjects, was found. However, the data suggested that frying with low to medium heat for less than 10 minutes might produce lower nitrosamine levels.

The goal of our work was to (1) estimate the nitrosamine content of cooked bacon purchased and prepared by consumers during routine meal preparation, (2) estimate a daily N-nitrosamine exposure, and (3) to estimate the volatile N-nitrosamine content of the fried-out fat.

MATERIALS & METHODS

Safety

N-nitrosamines are potent animal carcinogens and must be handled with appropriate safety precautions.

Reagents

Dichloromethane (DCM) and water were redistilled in glass and tested for artifacts. Nitrosamine standards were obtained commercially (Aldrich or Sigma) except N-nitrosothiazolidine (NTHZ) which was synthesized (Pensabene et al., 1972). All other reagents were analytical grade or better and tested for artifacts.

N-nitrosamine analyses

Cooked bacon samples were frozen in liquid N_2 and blended to a powder (Waring Blender Model 31BL92). Fried-out fat was melted (30°C) and stirred prior to analysis. All samples were distilled by a modified vacuum mineral oil procedure (Hotchkiss et al., 1985). Addition of 2,6-dimethylmorpholine to samples failed to produce N-nitrosodimethylmorpholine. Samples were spiked with N-nitrosodipropylamine (NDPA) at 7 μg/kg; recovery averaged 96% and 95% (s = 4.9 and 5.1) for the rasher and fried-out fat portions, respectively.

Samples were quantified by gas chromatography-Thermal Energy Analyzer GC-TEA (Fine et al., 1975) against external standards. GC-TEA conditions were as follows: Column, 3M × 0.32 cm o.d. Nickel packed with 10% Carbowax 20M on 80/100 Chromosorb WHP; carrier gas, He, 25 ml/min. Temperatures: injector, 190°C; column, 150°C; interface, 175°C pyrolyzer; 525°C; trap, −150°C. TEA pressure; 2.2 mm Hg. Nitrosamine identity was confirmed by GC-MS (Hewlett Package 5995C) in a manner similar to Hotchkiss et al. (1980).

Working standards were made routinely to insure consistency. The TEA response was monitored daily and showed little variation (the coefficient of variation for the NPYR peak was 4.4% over the entire study).

Sample collection

An interviewer approached customers of a cooperating local grocery store in July 1984 as they were leaving the bacon display case. The interviewer asked each customer to participate in a study concerning

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Table 1—Volatile N-nitrosamine content of consumer purchased and cooked bacon and fried-out fat[a,b]

Sample	Brand	Volatile N-nitrosamines			
		Rasher/fried-out fat			
		NDMA	NPIP	NPYR	NTHZ
100	B	tr[c]/tr	nd[d]/1.0	0.7/1.8	6.4/tr
102	B	2.7/5.9	nd/tr	12/22	8.6/nd
104	A	4.3/1.9	nd/nd	8.7/11	7.8/5.7
106	A	6.3/1.1	nd/tr	33/63	18/8.3
108	D	0.8/0.7	nd/tr	8.7/22	nd/nd
110	C	1.8/4.4	nd/3.0	22/45	5.5/tr
112	F	tr/1.7	nd/nd	4.6/17	nd/nd
114	D	0.8/1.0	nd/nd	11/14	4.1/5.4
116	N	4.3/3.6	nd/tr	34/76	15/10
118	P	tr/nd	nd/nd	tr/tr	nd/nd
120	G	2.7/12	1.3/3.0	37[f]/56[f]	6.0/nd
122	A	6.4/4.8	tr/1.7	19/27	13/3
124	L	23/4.3	5.7/5.0	20/8.1	19/nd
126	B	0.9/2.7	nd/1.2	16/81	12/14
130	A	2.3/8.5	1.8/2.2	25/54	tr/17
132	J	nd/tr	nd/nd	1.5/4.3	nd/nd
134	A	20/8.8	3.0/2.3	75[f]/54	30/6.9
136	N	13/7.1	tr/2.2	29/52	19/7.3
138	D	1.4/nd	nd/nd	4.8/nd	4.8/nd
140	E	1.2/3.5	nd/nd	4.6/6.0	13/nd
142	N	1.0./20	nd/3.0	8.0/13	5.3/nd
144	A	6.0/tr	nd/nd	7.0/7.0	5.0/tr
146	A	5.7/9.7	nd/2.0	4.6/29	7.7/tr
148	O	0.9/tr	tr/0.9	22[f]/9.1	19/tr
150	A	2.2/4.3	tr/1.9	22/46	20/7.0
152	A	2.9/4.0	nd/nd	5.5/29	5.0/3.6
154	E	0.5/1.2	nd/1.3	2.6/15	nd/nd
156	H	2.6/5.0	nd/nd	nd/nd	11/6.7
160	D	1.7/3.2	tr/tr	5.9/27	tr/nd
162	G	1.4/17	nd/6.2	12/170[f]	4.1/4.5
164	L	2.9/2.0	nd/1.3	15[f]/52	14/8.0
166	I	5.0/16	nd/1.9	18/27	13/nd
168	C	0.9/0.7	nd/nd	tr/tr	nd/nd
170	K	4.8/1.1	nd/1.9	4.3/27	4.9/5.2
172	Q	1.0/4.0	nd/tr	9.2/17	6.6/nd
174	B	1.5/0.6	tr/tr	8.6/11	12/7.0
180	M	9.4/9.4	1.3/3.0	23/28	20/4.4
182	A	0.9/2.0	nd/nd	0.7/9.7	6.7/5.2
184	D	10/33	9.2/10	130[f]/120[f]	11/tr
	$\bar{X}$[e]	4.0/5.3	0.7/1.5	17/32	8.9/3.4
	s	5.1/6.7	1.7/2.0	23/35	7.3/4.3

[a] μg/kg, not corrected for recovery
[b] recovery of internal spike (NDPA, 7 μg/kg) averaged >95%
[c] trace (<0.3 μg/kg)
[d] not detected
[e] calculated assuming tr = 0.3 μg/kg and nd = 0.1 μg/kg
[f] confirmed by GC-MS

the chemical composition of bacon. Forty-three of 51 customers approached agreed to participate. Each participant was mailed two coded containers (Nalgene 2118-0004), and a detailed questionnaire identified with a corresponding code. The instructions requested participants to cook six extra slices of the same bacon normally consumed in the household during the routine preparation of a meal containing bacon. They were told to use any brand of bacon, cook it in a routine fashion and drain the slices if that was part of their normal cooking method. Participants were asked to put the cooked slices in one container and the fried-out fat into the other. Samples were to be refrigerated or frozen, the questionnaire completed, and the interviewer called. The samples and questionnaires were picked up at the home within three days. Each participant was given $10. Samples were then stored at 4°C until analysis. Thirty-nine samples/questionnaires were returned.

Survey questionnaire

The seven-page questionnaire asked each participant about the cooking method used for the samples, the brand of the submitted samples, the amount and frequency of bacon consumption for each household member, the use of the fried-out fat and their personal attitudes about eating bacon.

RESULTS & DISCUSSION

THE VOLATILE N-nitrosamine content of the 39 rasher and fried-out fat samples obtained from the participants which represented 17 brands are shown in Table 1. All samples except No. 118 had measurable levels of one or more apparent volatile nitrosamines. Sample No. 118 was reported by the participant to be a brand which had "no nitrites added." The average NPYR content of all samples was 17 μg/kg. Thirty-six percent of the rashers had a NPYR content >17 μg/kg. Four (10%) of the rashers had concentrations 2× the action level. NTHZ was the second highest nitrosamine in average concentration in rashers followed by N-nitrosodimethylamine (NDMA) and N-nitrosopiperidine (NPIP). NPYR was found in quantifiable amounts with the greatest frequency.

Our data represent households containing 103 individuals as reported by the respondents. We calculated that the average consumption of rasher was 0.833 slices/day/person based on the reported frequency of consumption and number of slices consumed per sitting for each household member.

The average mass of a slice of fried bacon was estimated to be 5.3g based on our experience in frying and weighing locally purchased bacon (n = 93, s = 1.7; Hotchkiss and Vecchio, 1985). Using these values and the average nitrosamine content of the rashers (Table 1), we calculated the following daily volatile nitrosamine exposure (ng/person/day) from bacon for the respondents and their families: NDMA, 18; NPIP, 6.6; NPYR, 76; NTHZ, 40. This represents a total volatile N-nitrosamine exposure of 1.4 nmole/person/day. Fifteen percent of the respondents indicated that they consumed from 1.7 to 3.4 slices/day. For this group, the average NPYR exposure was 153 to 306 ng/person/day.

The Committee on Nitrite and Alternative Curing Agents in Food (National Research Council, 1981) estimated that the daily dietary exposure (U.S.) to NPYR from fried bacon was 170 ng/person/day. This was based on the incorrect assumption that annual bacon production equals consumption; approximately ⅔ to ¾ of the weight of raw bacon is lost during frying. They assumed the NPYR content of consumed bacon to be 5 μg/kg and that fried bacon was consumed by ¼ of the U.S. population. Correcting their annual bacon production figures for losses during frying, gives a daily fried bacon consumption of 8.5 g/person/day. Our respondents reported a consumption of 4.4 g/person/day. Our data suggests that the committee overestimated NPYR exposure from fried bacon of 94 ng/person/day. However, when the total mass of the four volatile nitrosamines found is considered, our data suggests 140 ng/person/day exposure.

Our NPYR estimate is approximately 56 ng/person/day higher than that of the American Meat Institute's (Wilson, 1985) 20 ng/person/day. They calculated an average cooked bacon consumption of 2.3 g/person/day based on annual bacon production figures and a 28% cooking yield. They assumed that the entire population of the U.S. consumed bacon. This gives just under one-half our per capita estimate for bacon consumption. They estimated that bacon contains an average of 10.6 μg NPYR/kg while we used the 17 μg/kg found in our survey.

Using these data to calculate the contribution of bacon to overall nitrosamine exposure may not be justified. There is evidence that the nitrosamine content of the U.S. diet is decreasing (Havery and Fazio, 1985) and accurate estimates of endogenous nitrosamine synthesis are not possible at present. Our data do indicate, however, that fried bacon results in an exposure to only minute amounts of N-nitrosamines which are a very small fraction of the exposure from other known sources such as the workplace (National Research Council, 1981), tobacco (Hecht et al., 1983) or cosmetics (Speigelhalder and Preussmann, 1984). The elimination of nitrosamines from fried bacon would result in only a very small reduction in total exposure.

The NPYR content of the rashers in descending order and the cooking method as reported by the respondents are shown in Table 2. No correlation between brand, reported degree of doneness, or draining the fat was found. All participants reported frying the bacon with the exception of two microwaved and one oven baked samples. The NPYR content of these three samples was well below the mean. Whether or not a cold or

Table 2—Bacon cooking procedures as reported by survey participants

Sample	NPYR[a] rasher	Cooking[b] method	No. of turnings	Drain[c] blot	Doneness[d]	Brand
184	130	F C S_{ml} U	2	N Y	WD	D
134	75	F C S_m U	3–4	N Y	VWD	A
120	37	F P E_{350} –[e]	1	Y Y	WD	G
116	34	F P S U	3	N Y	WD	N
106	33	F C – U	2	Y Y	WD	A
136	29	F P S_{mh} U	1	Y Y	WD	N
130	25	F P S_{mh} C_o	3	N Y	WD	A
180	23	F C S_h U	2	N Y	WD	M
150	22	F C S_m U	1	Y Y	WD	A
110	22	F C S_m U	–	N Y	WD	C
148	22	F P S_{ml} C_o	8–10	Y Y	WD	O
124	20	F C S_m U	2	N Y	WD	L
122	19	F P S_m U	1	N Y	WD	A
166	18	F C S_m U	–	N N	WD	I
126	16	F C S_{ml} U	8–10	Y Y	WD	B
164	15	F P S_l U	3	N N	WD	L
162	12	F C S_- U	4	N Y	M	G
102	12	F C S_{ml} U	8	N Y	WD	B
114	11	F C S_- U	4	Y N	M	D
172	9.2	F P S_m U	4	N Y	WD	Q
104	8.7	F C S_{350} U	2–3	Y Y	WD	A
108	8.7	F C S_l U	6	N Y	WD	D
174	8.6	F P E_{250} C_o	1	N Y	WD	B
142	8.0	F C S_m C_o	4	N Y	M	N
144	7.0	F C S_{ml} U	3	N Y	WD	A
160	5.9	F C S_l U	6–7	N Y	WD	D
152	5.5	F C E_{260} U	4	N Y	WD	A
138	4.8	M F_l 4 min	0	– N	VWD	D
112	4.6	F P S_{mh} U	4	N Y	WD	F
140	4.6	O_{450} E 11 min	0	– Y	WD	E
146	4.6	F C S_{ml} U	4	N N	VWD	A
170	4.3	F C S_m U	3	N Y	WD	K
154	2.6	F C S_m U	3–4	Y Y	VWD	E
132	1.5	F C S_{ml} U	–	– Y	WD	J
182	0.7	F C S_m U	4	N Y	WD	A
100	0.7	F C S_{ml} U	2	Y Y	VWD	B
168	TR	F P S_l U	4	Y Y	M	C
118	TR	F C S_l C_o	–	N Y	VWD	P
156	ND	M F_l 5 min	1	– Y	—	H

[a] μg/kg

[b] F, fried; M, microwaved; O, oven baked; C/P, cold or preheated pan; S/E, stove top or electric pan with setting (l, low; m, medium, h, high) or temperature (°F); C_o/U, covered or uncovered; F_l, full power (microwave).

[c] Draining fat between fryings; Blotting fat from the edible portion; Y = yes, N = No.

[d] R, rare (soft, limp, fat remaining); M, medium (soft, some fat remaining); WD well done (crisp, not much fat remaining); VWD (dark, very crisp).

[e] Question was not answered by participant.

preheated pan was used made no apparent difference in the NPYR content of the rasher.

The heat setting used to fry the bacon tended to be higher for those rashers with a NPYR content above the median. Only four respondents with NPYR contents below the median reported heat settings of medium or higher while nine of the group above the median used a medium or higher setting when frying. Pensabene et al. (1974) showed that the degree of doneness was less important than the temperature of the pan in determining the NPYR content of the rasher. They fried randomized bacons to the same degree of doneness over pan temperatures of 99° to 204°C and times of 105 to 4 minutes. The combination of higher temperatures for shorter times resulted in more NPYR. While nearly all participants reported cooking their bacon to well done (Table 2), the samples above the median in NPYR, in general, were fried with higher heat settings.

Several laboratory studies have shown that the volatile nitrosamine content of fried-out bacon fat is higher than the corresponding edible portion (Pensabene et al., 1980). Fried-out fat from our consumer cooked bacon likewise had a higher nitrosamine content by an average of 1.9 fold for NPYR. We have previously shown that frying noncured foods in fried-out fat resulted in the formation of volatile nitrosamines; as much as 20 μg of NDMA when 100 g of fish was fried in 60 mL fried-out fat (Hotchkiss and Vecchio, 1985). The majority of the volatile nitrosamines are carried off with the vapor and only trace amounts remain in the cooked edible portion. A majority (69%) of the participants indicated that they used the fat as a frying oil although only 18% said they saved the fried-out fat for use at a later time. Frying eggs was the most common (62%) use of the fat followed by frying potatoes (18%), vegetables (10%) and liver, bread, and pancakes (5% each). Seven respondents reported using fried-out fat as an ingredient.

Our data indicate that, at least for our respondents, the volatile N-nitrosamine content of bacon as purchased and prepared by consumers is higher than indicated by the FSIS bacon monitoring program. However, fried bacon represents a minor exposure to N-nitrosamines when compared to other known exposures.

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Effects of Different Chilling Methods on Hot Processed Vacuum Packaged Pork

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ABSTRACT

Longissimus muscle (loin section) was removed from each side of 24 pork carcasses within 20 min postmortem. Each muscle was divided in half (N = 96 sections), vacuum packaged and allotted to one of four treatments: (1) normal chilling, (2) propylene glycol immersion, (3) crust freezing, or (4) high temperature conditioning and four storage intervals (N = 6) or 0, 7, 14 or 28 days. Rapid chilling with propylene glycol increased ($P<0.05$) purge. Shear force values were increased when loins were rapidly chilled ($P<0.05$) but few sensory differences were observed. Off-odor scores increased ($P<0.05$) with increasing storage time. Normal chilling resulted in lighter muscle color scores than rapidly chilled loin sections. Rapid chilling does not have a beneficial effect on palatability or shelf life of hot-processed fresh pork.

INTRODUCTION

VACUUM PACKAGING has been shown to extend the shelf life of fresh pork (Weakley et al., 1986; Smith et al., 1974; Ramsey et al., 1973). In addition, vacuum packaging reduces shrinkage, surface discoloration, and bacteria counts of fresh pork (Smith et al., 1974). Weakley et al. (1986) reported that vacuum packaged pork cuts could be stored at 4°C for up to 28 days and produce satisfactory retail cuts. Few differences in palatability or storage characteristics were observed between conventional and vacuum packaged pork.

Hot processing of pork carcasses for fresh cuts can reduce in-plant holding time up to 21 hr (Mandigo, 1967, 1968). In addition, significant reductions in energy costs are attained because excess fat and bone are not chilled (Reagan, 1983b). A combination of hot processing and vacuum packaging could potentially result in several advantages and would enable processors to utilize alternative and/or more efficient methods of chilling.

Rapid chilling methods have the potential advantage of improving pork color. Pale, soft, exudative pork can result from high temperatures, rapid postmortem glycolysis, and early onset of rigor mortis in the muscle (Briskey, 1964; Wismer-Pedersen, 1959). Rapid chilling reduces the development of pale, soft, exudative pork (Borchert and Briskey, 1964).

The present study was designed to evaluate the effects of different chilling methods on the palatability and storage traits of vaccum packaged hot processed fresh pork.

MATERIALS & METHODS

TWENTY-FOUR market weight (104 kg) crossbred Hampshire-sired barrows of similar genetic background were slaughtered using conventional skinning methods. Boneless loin sections were obtained by removal (within 20 min postmortem) of the longissimus muscles from the last lumbar vertebrae to the third rib. Each loin section was trimmed to 0.6 cm subcutaneous fat thickness and divided into two equal segments. Loin sections were weighed and vacuum packaged in heat-shrinkable vacuum package bags (Cryovac — B650 bags with an O_2 transmission rate of 30 $mL/m^2/24$ hr) using a heat seal vacuum packaging machine (Multivac AGW — maximum vacuum time was utilized). After packaging, vacuum bags were heat shrunk by immersion in 74°C water for 1–2 sec. The four loin sections from each of six carcasses (N = 24) were randomly assigned to chilling treatments: normal chilling (2°C cooler), propylene glycol (immersion in 33% solution of propylene glycol for 1 hr at -20 ± 2°C), crust freezing (-30 ± 2°C blast freezer for 1 hr) and high temperature conditioning (17°C for 3 hr). Propylene glycol immersion for 1 hr at the described temperatures resulted in crust freezing of the loin sections. After chilling, loin sections were held in a cooler (2°C) for storage intervals of 0, 7, 14, or 28 days (Fig. 1). At the end of each storage period, loin sections (n = 6/treatment) were removed and prepared for subsequent evaluation.

In a preliminary study, temperature decline was monitored for the various chilling treatments. At 1 hr postmortem, the internal temperatures of the normal chill (NC), propylene glycol (PG) and crust freezing (CF) were 24°, 11° and 13°C, respectively. High temperature conditioning (HT) of loin sections for 3 hr postmortem resulted in an internal temperature of 22°C.

Shrinkage data were obtained by weighing each loin section prior to vacuum packaging and weighing upon removal from the bag. Total plate counts were obtained from one loin of each carcass prior to packaging. At the end of the appropriate storage interval, bacterial counts were obtained from each loin section. Surface areas of 10 cm^2 were cut from the surface of loin sections using an aluminum template. Samples were placed in a stomacher bag with 100 mL 0.01% peptone diluent. Samples were blended for 1 min, appropriate dilutions made and samples were pour plated using Plate Count Agar. Colony number was enumerated after 48 hr incubation at 35°C (Messer et al., 1980). At the end of the appropriate storage intervals, a six-member, experienced panel evaluated samples within 10 min of opening the bag for incidence of off-odor using a four-point scale (1 = no off-odor, 4 = very strong off-odor).

After each storage interval, loin sections were fabricated into six consecutive boneless chops (3.2 cm thick) from the cut surface of the longissimus where the two loin sections were separated. The cut surface of the longissimus where the loin sections were divided was trimmed and used for microbial evaluation. Chops 1, 3, and 4 were wrapped in wax-lined freezer paper, frozen (-30°C) and held for subsequent evaluation. Chop 1 was removed from the freezer, thawed 24 hr (4°C) and cooked on a Farberware grill to an internal temperature of 75°C (American Meat Science Association and National Live Stock and Meat Board, 1978). Internal temperature was monitored using copper constantan thermocouples and a Campbell CR-5 temperature recorder. Chops were allowed to cool to room temperature and three cores (1.27 cm diameter) were removed parallel to the muscle fiber orientation. Each core was sheared twice using an Instron with a Warner-Bratzler shear head attachment (measurements were recorded in kg).

Chops 2 and 5 were packaged using plastic foam trays with a bottom blotter pad and overwrapped with a high O_2 transmission polyvinylchloride film. Samples were allowed to bloom for at least 30 min after cutting and color values (L, a, b) were evaluated (4 readings/sample) using a Hunter Laboratories D25-9 Color Difference meter, standardized using a L = 92.42, a = -1.09, b = 1.60 white color chip. Samples were then placed in a lighted retail case (4°C) and evaluated daily by a six member, experienced panel. The retail case was continuously lighted using General Electric cool white fluorescent bulbs with 10.53 Lux intensity. The panel evaluated lean color using a 5-point scale where 1 = pale and 5 = dark (Wisconsin Pork Quality Standards, 1963); surface discoloration using a 7-point scale where 1 = no surface discoloration and 7 = complete discoloration (Jeremiah

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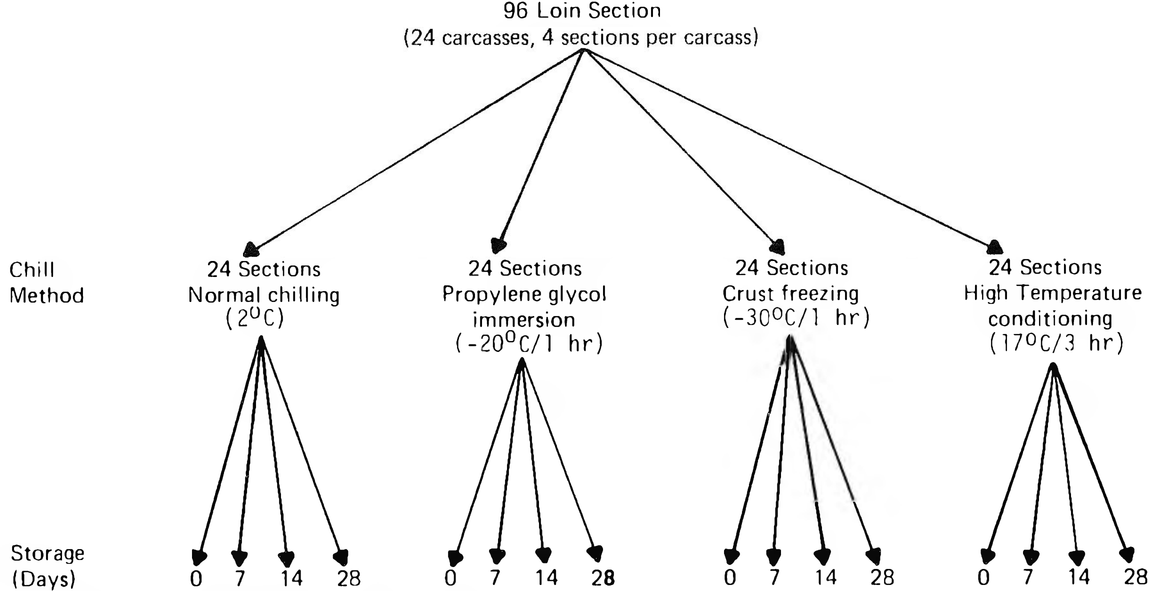

Fig. 1—Experimental design for chilling treatments of hot processed pork.

Table 1—Mean values for storage characteristics of hot processed, vacuum packaged fresh pork using various chilling methods

Storage interval	Chilling treatment NC[a]	PG	CF	HT
0 Day				
Shrinkage (%)	$^{y}0.73^{AB}$	$^{z}0.69^{AB}$	0.84^{A}	$^{y}0.52^{B}$
Bacteria count prior to packaging (Log 10/cm²)	2.26	2.26	2.26	2.26
Bacteria count after storage (Log 10/cm²)	$^{y}2.26$	$^{y}2.26$	$^{y}2.26$	$^{y}2.26$
Off-odor[b]	$^{y}1.12$	$^{z}1.02$	$^{z}1.12$	$^{z}1.05$
7 Day				
Shrinkage (%)	$^{y}0.65^{C}$	$^{yz}1.96^{A}$	1.29^{B}	$^{y}0.63^{C}$
Bacteria count prior to packaging (Log 10/cm²)	2.11	2.11	2.11	2.11
Bacteria count after storage (Log 10/cm²)	$^{z}1.35^{B}$	$^{z}1.22^{B}$	$^{y}2.55^{A}$	$^{y}1.98^{AB}$
Off-odor[b]	$^{y}1.03$	$^{z}1.17$	$^{z}1.22$	$^{z}1.06$
14 Day				
Shrinkage (%)	$^{y}0.71^{B}$	$^{xy}3.61^{A}$	2.29^{AB}	$^{xy}1.16^{B}$
Bacteria count prior to packaging (Log 10/cm²)	1.84	1.84	1.84	1.84
Bacteria count after storage (Log 10/cm²)	$^{yz}1.63^{AB}$	$^{z}1.21^{B}$	$^{y}1.86^{AB}$	$^{y}2.11^{A}$
Off-odor[b]	$^{x}1.42^{B}$	$^{y}1.59^{AB}$	$^{y}1.52^{AB}$	$^{y}1.73^{A}$
28 Day				
Shrinkage (%)	$^{x}1.57^{B}$	$^{x}4.29^{A}$	1.99^{B}	$^{x}1.71^{B}$
Bacteria count prior to packaging (Log 10/cm²)	2.12	2.12	2.12	2.12
Bacteria count after storage (Log 10/cm²)	$^{x}4.75$	$^{x}4.87$	$^{x}5.58$	$^{x}5.33$
Off-odor[b]	$^{x}1.64^{B}$	$^{x}2.08^{AB}$	$^{x}1.75^{AB}$	$^{x}2.19^{A}$

^{ABC} Means in the same row for the same trait with different superscripts are different (P<0.05).
^{xyz} Means in the same column for the same trait with different superscripts are different (P<0.05).
[a] NC-normal chill (2°C); PG-propylene glycol chill (−20°C); CF-crust freezing (−30°C); HT-high temperature (17°C).
[b] Means based on evaluations by a six member, experienced panel using a scale of 1–4 (1 = no off-odor; 2 = slight off-odor; 3 = moderate off-odor; 4 = very strong off-odor).

and Greer, 1982), and overall appearance using a 9-point scale where 1 = extremely desirable and 9 = extremely undesirable.

After chops 3 and 4 had been cut and frozen, they were used for sensory evaluation. Panelists consisted of faculty and staff from the program who had previous sensory experience with pork products. They were familiarized with the ballot prior to evaluating palatability traits. Chops were cooked using the same procedures described for Chop 1. Samples were served warm to a six-member, experienced sensory panel. Samples were evaluated for juiciness, tenderness, pork-flavor intensity and off-flavor intensity where 0 = extremely dry, tough, bland or intense and 15 = extremely juicy, tender, intense or bland using an unstructured scale with anchors at each endpoint and a centerline. Numerical values ranging from 0–15 were assigned to each response by measuring the distance from 0 to the response.

Chop 6 was used to determine ultimate pH values, sarcomere length and for proximate analysis. Proximate analysis samples were trimmed of all fat and epimysial connective tissue, ground twice, and duplicate 5 g samples were analyzed. Percent fat was determined by extraction with warm chloroform-methanol (2:1) (Riss et al., 1983). The samples for sarcomere length determination were obtained by removing a 1.27 cm diameter core of muscle from the center of the longissimus muscle. Sarcomere length was determined by homogenizing 3g of muscle in 25 mL of 0.25M sucrose for 20–40 sec in a Virtis "23" homogenizer. Average sarcomere lengths were determined from measurements of 25 different fragments using an eyepiece micrometer calibrated to a stage micrometer using a Zeiss phase contrast microscope.

The data were analyzed using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 1982). Differences between means were analyzed by Duncan's Multiple Range Test (Duncan, 1955; SAS, 1982).

RESULTS & DISCUSSION

A COMPARISON of the storage characteristics of the four chilling treatments (NC, PG, CF and HT) on fresh, hot-boned, vacuum packaged pork is shown in Table 1. Shrinkage values were significantly higher for PG chilled loins after 7, 14 and 28 days with the exception of CF on day 14. NC and HT treatments had less (P<0.05) shrinkage than CF at day 7. Rapid chilling procedures used in this study resulted in some surface freezing which may have contributed to increased purge and thus more shrinkage. Increased purge losses were also reported for rapid chilling procedures in a review (Reagan,

Table 2—Sensory characteristics and shear force values of hot processed, vacuum packaged fresh pork using various chilling methods

Storage interval	Chilling treatment			
	NC[a]	PG	CF	HT
0 Day				
Shear value (Kg)	$^{x}4.23^{B}$	4.81^{B}	$^{x}6.16^{A}$	$^{x}4.69^{B}$
Juiciness[b]	9.01	$^{x}10.02$	9.51	8.64
Tenderness[b]	$^{x}6.95$	$^{y}6.37$	$^{z}6.10$	$^{z}6.54$
Flavor intensity[b]	9.66^{AB}	$^{y}9.05^{B}$	10.27^{A}	8.91^{B}
Off-flavor[b]	12.48	$^{xy}13.29$	12.98	$^{y}12.31$
7 Day				
Shear value (Kg)	$^{y}2.86^{C}$	4.39^{AB}	$^{y}4.81^{A}$	$^{y}3.60^{BC}$
Juiciness[b]	9.92	$^{xy}9.23$	9.03	8.01
Tenderness[b]	$^{y}10.58$	$^{xy}7.93$	$^{y}9.59$	$^{y}9.28$
Flavor intensity[b]	9.91	$^{xy}9.68$	10.18	10.18
Off-flavor[b]	13.34^{AB}	$^{xy}12.94^{B}$	13.35^{AB}	$^{x}14.05^{A}$
14 Day				
Shear value (Kg)	$^{y}2.82^{C}$	4.18^{A}	$^{y}3.63^{AB}$	$^{y}2.96^{BC}$
Juiciness[b]	10.21	$^{x}10.20$	9.71	10.26
Tenderness[b]	$^{y}11.83$	$^{x}11.17$	$^{x}11.78$	$^{x}12.06$
Flavor intensity[b]	11.08	$^{x}10.96$	10.60	10.60
Off-flavor[b]	13.43	$^{x}13.78$	12.39	$^{xy}13.15$
28 Day				
Shear value (Kg)	$^{y}2.74^{C}$	4.31^{A}	$^{y}3.72^{AB}$	$^{y}3.36^{BC}$
Juiciness[b]	9.58^{A}	$^{y}8.03^{B}$	9.11^{AB}	9.23^{AB}
Tenderness[b]	$^{y}11.02$	$^{xy}9.84$	$^{xy}10.37$	$^{xy}11.13$
Flavor intensity[b]	10.18	$^{xy}10.21$	10.36	10.63
Off-flavor[b]	12.54	$^{y}12.43$	13.29	$^{y}12.17$

^{ABC} Means in the same row for the same trait with different superscripts are different ($P<0.05$).
^{xyz} Means in the same column for the same trait with different superscripts are different ($P<0.05$).
[a] NC-normal chill (2°C); PG-propylene glycol chill (−20°C); CF-crust freezing (−30°C); HT-high temperature (17°C).
[b] Means based on evaluations by a six-member, experienced panel using a scale of 1-15 (1 = extremely dry, tough, bland, intense; 15 = extremely juicy, tender, intense, none).

Table 3—Mean values for meat characteristics of hot processed, vacuum packaged fresh pork using various chilling methods

Storage interval	Chilling treatment			
	NC[a]	PG	CF	HT
0 Day				
pH value	$^{y}5.22$	$^{y}5.33$	$^{y}5.28$	$^{z}5.25$
Sarcomere length (μm)	$^{xy}1.90$	1.80	$^{y}1.86$	1.88
Fat (%)	$^{x}6.21^{A}$	$^{xy}4.48^{B}$	$^{x}4.59^{B}$	$^{x}6.06^{A}$
7 Day				
pH value	$^{x}5.55$	$^{xy}5.43$	$^{x}5.54$	$^{xy}5.49$
Sarcomere length (μm)	$^{y}1.83$	1.78	$^{y}1.85$	1.90
Fat (%)	$^{x}5.72$	$^{xy}5.38$	$^{x}4.54$	$^{yz}4.11$
14 Day				
pH value	$^{xy}5.38^{A}$	$^{xy}5.42^{A}$	$^{y}5.28^{B}$	$^{yz}5.28^{B}$
Sarcomere length (μm)	$^{x}2.00^{A}$	1.70^{C}	$^{y}1.89^{AB}$	1.81^{BC}
Fat (%)	$^{x}6.60^{A}$	$^{x}6.45^{AB}$	$^{x}5.71^{BC}$	$^{xy}5.52^{C}$
28 Day				
pH value	$^{x}5.48^{B}$	$^{x}5.55^{AB}$	$^{x}5.59^{A}$	$^{x}5.57^{AB}$
Sarcomere length (μm)	$^{xy}1.94^{AB}$	1.81^{C}	$^{x}2.05^{A}$	1.84^{BC}
Fat (%)	$^{y}3.60$	$^{y}3.86$	$^{y}3.06$	$^{z}3.64$

^{ABC} Means in the same row for the same trait with different superscripts are different ($P<0.05$).
^{xyz} Means in the same column for the same trait with different superscripts are different ($P<0.05$).
[a] NC-normal chill (2°C); PG-propylene glycol chill (−20°C); CF-crust freezing (−30°C); HT-high temperature (17°C).

1983b). Shrinkage values reported for the PG treatment on days 14 and 28 are excessive (Reagan, 1983b).

Incidence of off-odor increased ($P<0.05$) with increased storage time. High temperature conditioning resulted in higher ($P<0.05$) off-odor scores than NC on days 14 and 28. Total bacteria count increased with increasing storage intervals. CF resulted in higher total plate counts than NC and PG loin sections on day 7 and on day 14, PG had lower total plate counts than HT cuts. Overall, bacterial levels were low after 28 days of storage (4.75 to 5.58 log $10/cm^2$).

Warner-Bratzler shear (WBS) values were significantly higher for rapid-chilling treatments after storage for 7, 14 and 28 days (Table 2). CF had higher ($P<0.05$) WBS values than NC at all storage periods and higher values than HT on days 0 and 7. WBS values from PG treatments differed from NC on days 7, 14 and 28, and from HT treatments after 14 and 28 days of storage. A significant tenderness improvement was observed for the NC, CF and HT cuts between 0 and 7 days of storage which is consistent with the reports of Gould et al. (1965) and Harrison et al. (1970). Decreases in tenderness due to rapid chilling of pork have been reported by Weiner et al. (1966), Marsh et al. (1972) and Reagan (1983a). These reports indicate rapidly chilled or partially frozen loin chops exhibit cold-shortening, although the effects of cold-shortening in pork may not elicit the same magnitude of tenderness loss seen in beef (Marsh et al., 1972). Sensory traits varied little within chilling treatment or storage interval. This finding is in agreement with Hinnergardt et al. (1973) and Miller et al. (1984) who reported few differences in sensory properties of rapid chilled pork. In the present study, muscle samples were randomly allotted to chilling treatments in an effort to prevent confounding effects of differential rates of rigor development due to location in the longissimus muscle.

Mean values for longissimus characteristics are shown in Table 3. Mean pH values did not differ consistently between treatments or storage intervals. No differences in sarcomere lengths were observed between treatments on days 0 and 7. Cold shortening may be expected in some of the chilling treatments used in this study (rapid chilling within 30 min postmortem); however, on storage days 0 and 7 statistical differences

Table 4—Mean values for lean color characteristics of hot processed, vacuum packaged fresh pork using various chilling methods

	Chilling treatment			
Storage interval	NC[a]	PG	CF	HT
0 Day				
Colorimeter L value[b]	$^{z}37.88^{A}$	$^{y}36.48^{BC}$	$^{z}35.58^{C}$	$^{z}37.15^{AB}$
Colorimeter a value[c]	4.26	$^{y}2.42$	3.41	4.46
Colorimeter b value[d]	$^{y}5.28^{A}$	$^{y}4.08^{B}$	$^{z}4.63^{AB}$	$^{y}5.49^{A}$
7 Day				
Colorimeter L value[b]	$^{yz}40.28^{A}$	$^{y}37.91^{B}$	$^{y}38.87^{AB}$	$^{yz}38.73^{AB}$
Colorimeter a value[c]	4.12^{A}	$^{x}3.61^{AB}$	3.41^{B}	3.40^{B}
Colorimeter b value[d]	$^{x}6.80^{A}$	$^{x}5.98^{B}$	$^{y}6.23^{AB}$	$^{xy}6.49^{AB}$
14 Day				
Colorimeter L value[b]	$^{x}45.50^{A}$	$^{x}41.69^{C}$	$^{x}44.29^{AB}$	$^{x}43.26^{B}$
Colorimeter a value[c]	4.06	$^{x}3.72$	4.11	4.07
Colorimeter b value[d]	$^{x}7.41^{A}$	$^{x}6.84^{B}$	$^{x}7.49^{A}$	$^{x}7.53^{A}$
28 Day				
Colorimeter L value[b]	$^{y}42.40^{A}$	$^{x}40.54^{B}$	$^{y}40.82^{B}$	$^{xy}40.81^{B}$
Colorimeter a value[c]	3.52^{AB}	$^{xy}3.13^{B}$	3.60^{A}	3.21^{AB}
Colorimeter b value[d]	$^{x}6.82^{A}$	$^{x}6.09^{B}$	$^{xy}7.04^{A}$	$^{x}6.82^{A}$

^{ABC} Means in the same row for the same trait with different superscripts are significantly different ($P<0.05$).
^{xyz} Means in the same column for the same trait with different superscripts are significantly different ($P<0.05$).
[a] NC-normal chill (2°C); PG-propylene glycol chill (−20°C); CF-crust freezing (−30°C); HT-high temperature (17°C).
[b] L = 100 = white; L = 50 = gray; L = 0 = black.
[c] $-a$ = green; $+a$ = red; a = gray.
[d] $-b$ = blue; $+b$ = yellow; b = 0 = gray.

Table 5—Mean values for lean color, surface discoloration and overall appearance of hot processed, vacuum packaged pork using various chilling methods

	Color[a]				Discoloration[a]				Appearance[a]			
	NC[b]	PG	CF	HT	NC[b]	PG	CF	HT	NC[b]	PG	CF	HT
0 Day												
Display day 1	3.00^{C}	3.21^{BC}	$^{x}3.67^{A}$	3.43^{AB}	$^{y}1.22$	$^{z}1.21$	$^{z}1.14$	$^{y}1.26$	$^{y}2.19$	$^{z}2.05$	$^{y}2.17$	$^{y}2.12$
Display day 2	$^{y}2.57^{B}$	$^{y}2.57^{B}$	2.98^{A}	3.07^{A}	1.74	$^{y}1.88$	1.69	$^{xy}1.76$	3.26^{A}	$^{z}3.21^{A}$	2.50^{B}	$^{y}2.67^{AB}$
Display day 3	$^{y}2.60^{C}$	2.70^{BC}	2.97^{AB}	3.03^{A}	2.07	$^{z}2.10$	$^{y}2.00$	$^{y}1.93$	3.83^{AB}	4.20^{A}	$^{y}3.17^{BC}$	$^{z}3.07^{C}$
Display day 4	2.74	$^{y}2.75$	3.06	3.03	2.36	$^{y}2.39$	$^{y}2.08$	$^{z}2.22$	4.03^{AB}	$^{y}4.36^{A}$	$^{y}3.67^{AB}$	$^{z}3.36^{B}$
7 Day												
Display day 1	3.03	3.07	$^{y}3.23$	3.13	$^{y}1.30$	$^{z}1.37$	$^{yz}1.27$	$^{y}1.23$	$^{xy}2.60^{AB}$	$^{y}2.93^{A}$	$^{y}2.40^{B}$	$^{y}2.23^{B}$
Display day 2	$^{xy}2.86^{B}$	$^{x}3.08^{AB}$	3.33^{A}	3.33^{A}	1.80	$^{xy}2.03$	1.83	$^{y}1.64$	3.25^{AB}	$^{yz}3.69^{A}$	3.00^{B}	$^{xy}2.86^{B}$
Display day 3	$^{x}2.92^{B}$	3.00^{B}	3.33^{A}	3.22^{AB}	2.05	$^{yz}2.36$	$^{y}2.16$	$^{y}2.03$	3.89	4.11	$^{xy}3.89$	$^{yz}3.80$
Display day 4	2.83^{B}	$^{xy}3.00^{AB}$	3.27^{A}	3.17^{A}	2.87	$^{xy}3.13$	$^{xy}2.80$	$^{yz}2.60$	4.43	$^{xy}4.77$	$^{xy}4.40$	$^{z}4.00$
14 Day												
Display day 1	2.85^{B}	3.16^{A}	$^{y}3.07^{A}$	3.12^{A}	$^{x}1.71^{B}$	$^{x}2.28^{A}$	$^{xy}1.59^{B}$	$^{xy}1.57^{B}$	$^{x}3.12^{B}$	$^{x}3.66^{A}$	$^{y}2.31^{C}$	$^{xy}2.50^{C}$
Display day 2	$^{xy}2.67^{B}$	$^{xy}3.00^{A}$	3.03^{A}	3.00^{A}	2.13^{AB}	$^{x}2.57^{A}$	1.93^{B}	$^{x}2.10^{AB}$	4.07^{AB}	$^{x}4.80^{A}$	3.03^{B}	$^{x}3.63^{AB}$
Display day 3	$^{xy}2.77^{B}$	3.00^{AB}	3.11^{A}	3.10^{A}	2.50	$^{x}3.33$	$^{xy}2.63$	$^{x}3.33$	4.73	5.37	$^{xy}4.33$	$^{xy}5.13$
Display day 4	2.69^{B}	$^{x}3.11^{A}$	2.97^{A}	3.03^{A}	2.89	$^{x}3.69$	$^{x}3.55$	$^{xy}3.64$	5.22	$^{x}5.80$	$^{x}5.75$	$^{y}5.47$
28 Day												
Display day 1	3.03	3.14	$^{xy}3.31$	3.28	$^{x}1.69$	$^{y}1.91$	$^{x}1.91$	$^{x}1.78$	$^{xy}2.91$	$^{x}3.61$	$^{x}3.19$	$^{x}3.08$
Display day 2	$^{x}2.97$	$^{x}3.08$	3.24	3.18	1.78	$^{xy}2.11$	2.14	$^{xy}1.98$	3.16^{B}	$^{xy}4.44^{A}$	3.44^{AB}	$^{x}3.72^{AB}$
Display day 3	$^{x}3.04$	3.06	3.15	3.25	2.54^{B}	$^{xy}2.92^{B}$	$^{x}3.29^{AB}$	$^{x}3.92^{A}$	4.21^{B}	4.96^{AB}	$^{x}4.83^{AB}$	$^{x}6.10^{A}$
Display day 4	3.08	$^{x}3.15$	3.16	3.19	2.72^{B}	$^{xy}3.00^{B}$	$^{x}3.72^{AB}$	$^{x}4.39^{A}$	4.17^{C}	$^{xy}5.36^{BC}$	$^{x}5.75^{B}$	$^{x}7.16^{A}$

^{ABC} Means in the same row for the same trait with different superscripts are different ($P<0.05$).
^{xyz} Means in the same column for the same trait with different superscripts are significantly different ($P<0.05$).
[a] Means based on the evaluations by a six-member, experienced panel (acceptability: 1 = extremely desirable, 9 = extremely undesirable; lean color: 1 = pale, 5 = dark, surface discoloration: 1 = no discoloration, 7 = complete discoloration).
[b] NC-normal chill (2°C); PG-propylene glycol chill (−20°C); CF-crust freezing (−30°C); HT-high temperature (17°C).

were not observed. On days 14 and 28, the sarcomere lengths of the PG treatment were shorter ($P<0.05$) than the NC, which is consistent with WBS differences. The samples used for sarcomere length determination were obtained from the center of the muscle (1.3 cm core). It is possible the center of the muscle would be warmer and not exhibit the same degree of cold shortening as the periphery of the muscle. Fat content differed between treatments on days 0 and 14 and between storage intervals. Differences between storage periods may be attributed to animal to animal variation and/or variation within the longissimus muscle. Differences in fat content between treatments may be attributed to variation within a muscle and/or differences in moisture loss between treatments.

Rapid chilling treatments resulted in lower "L" Hunter Colorimeter Readings indicating a darker colored lean (Table 4). PG loin sections had lower ($P<0.05$) L values than NC at all time periods; however, CF differed from NC only at days 0 and 28. PG treated loin sections consistently had the lowest b values ($P<0.05$). Both L and b values increased ($P<0.05$) with increased storage intervals.

After storage of the loin sections for various lengths of time, chops were cut and put in retail display for 4 days. Visual color evaluation after 0, 7 and 14-day storage intervals showed NC loin sections had lower color scores than other treatments (Table 5). Storage intervals did not consistently affect visual color score. The effect of rapid chilling on the color of hot processed pork agrees with the report of Borchert and Briskey (1964). However, high temperature conditioning resulted in darker colored pork than the NC chops.

Few differences were observed for surface discoloration (Table 5) among chilling treatments. As expected, surface discoloration values increased with storage time for PG, CF and HT treatments. Chops from the NC group differed between storage intervals at display day 1 but did not differ ($P>0.05$) between storage periods for display days 2, 3 and 4.

Overall appearance scores of the various chilling treatments

—Continued on page 765

Ultrastructure of Pork Liver after Freeze-Thaw Cycling and Refrigerated Storage

SUSAN B. JONES, ELIZABETH D. STRANGE, and BEVERLY E. MALEEFF

ABSTRACT

Pork liver was subjected to repeated freezing (−20°C) and thawing (+5°C) to simulate conditions of temperature abuse of frozen liver during commercial transport. Ultrastructure was compared to that of refrigerated pork liver. Liver cells deteriorated more with freezing and thawing (F-T) than with refrigerated storage (R-S). After one cycle F-T, hepatocyte organelles were damaged and cytoplasmic components appeared in the sinusoids. After four cycles F-T, membranes were extensively damaged and sinusoids contained organelles. The tissue organization was stable during six days of refrigerated storage, although cell structure deteriorated. Evidence from ultrastructure indicated that drip from F-T liver and R-S liver arose from different kinds of tissue damage.

INTRODUCTION

THE U.S. MEAT INDUSTRY supplies nearly one-third of the beef and pork variety meats (edible offal) in the European market, a valuable outlet for organ meats which have limited consumer appeal in the United States (Berry et al., 1982). Most liver is exported frozen because time in transit is typically 12–14 days. Transport of chilled, vacuum-packaged liver is also feasible if the transport-distribution-storage period is less than 15-16 days (Smith et al., 1983).

A recurring problem in exportation of frozen variety meats is deterioration of product quality during transit. Temperature fluctuations can produce thawing or cycles of thawing and freezing. Product damage is made apparent by leakage of tissue fluid and staining or deterioration of packaging. Other indices of product failure are excessive microbial flora, discoloration, and off-odors. Rejection rates of edible offal at port of entry ranged from 5–30% in 1978 (Turczyn, 1980), and the variety meat most rejected was liver. Prompt packaging and freezing and control of ambient temperature during transit are factors in maintaining product quality (Stiffler and Smith, 1983).

Partmann (1973) observed by light microscopy that large ice crystals produced in pork liver by slow freezing pushed hepatocyte chords apart and caused minor leakage from cells into the extracellular space. Strange et al. (1985a, b) reported that repeated freezing and thawing degraded liver differently than did refrigerated storage, based on protein solubility, isolability of liver cells, and collagen-staining properties. The purpose of this report is to present results of ultrastructure studies investigating tissue damage resulting from refrigerated storage versus freeze-thaw cycling.

MATERIALS & METHODS

WHOLE PORK LIVERS were obtained from a local slaughterhouse immediately after Federal inspection and removal of the bile duct and gall bladder and were transported on ice to the laboratory. Vacuum packaged 125–150g samples were stored at −20°C or +5°C within 3 hr of slaughter. Samples for freeze-thaw cycling were frozen at −20°C for 70 hr and then thawed at +5°C for 24 hr (one freeze-thaw cycle). Each subsequent freeze-thaw cycle was 24 hr at −20°C plus 24 hr at +5°C. Refrigerated liver samples were held at +5°C and each 24-hr period was called a cycle. Freeze-thaw (F-T) and refrigerated (R-S) samples with the same cycle number had equivalent time in refrigerated storage.

For electron microscopy, tissue was sampled fresh (within 3 hr of slaughter), after 1, 4, and 6 F-T cycles, and after 1, 4, and 6 days R-S. Small pieces (3-4 mm^3) of tissue were immersed in 2% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.35) at +4°C for 2 hr, then divided into smaller pieces and rinsed 3 × 30 min with 0.08M sodium cacodylate (pH 7.35) containing 3.4% sucrose. Tissue was post-fixed in 1% osmium tetroxide in 0.1M cacodylate buffer (pH 7.0) for 3 hr at room temperature. Fixed tissue was dehydrated in a graded ethanol series, including 1 hr *en bloc* staining with 2% uranyl acetate in 70% ethanol, and embedded in EMbed-812 (EM Sciences, Fort Washington, PA). Thin sections (60 nm) for transmission electron microscopy (TEM) were stained with uranyl acetate and lead citrate. For scanning electron microscopy (SEM), fixed tissue (approximately 3mm^3) was dehydrated in ethanol, rapidly frozen in liquid nitrogen, and cryofractured by pressure from a scalpel blade (Jones et al., 1976). Fractured tissue was transferred into ethanol, critical-point dried, secured to specimen mounts, and sputter-coated with 15-20 nm of gold-palladium alloy.

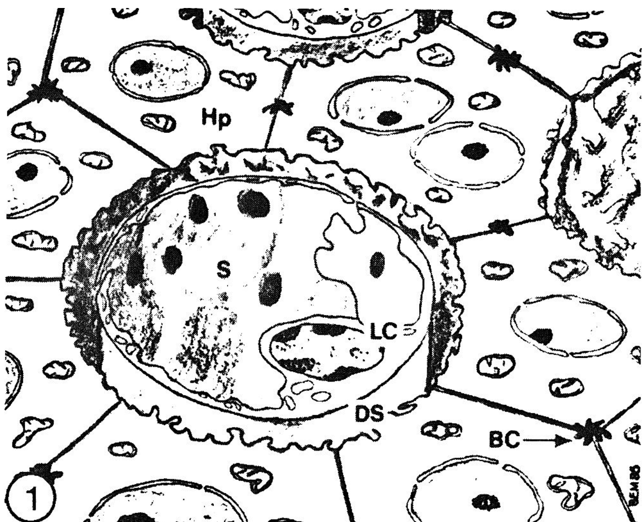

Fig. 1—Schematic of liver cell-sinusoid organization of liver parenchyma. (Adapted from Elias and Sherrick, 1969.) Hp = hepatocyte; S = sinusoid; LC = littoral cell; DS = Disse's space; BC = bile canaliculus.

RESULTS

LIVER PARENCHYMA is organized into columns of hepatocytes (liver cells) arranged around sinusoids, so that at least one face of each hepatocyte borders on a sinusoid (Fig. 1). Hepatocytes constitute 78% and the sinusoidal space (capillary bed), 18% of liver volume (Blouin et al., 1977). Nonhepatocyte liver cells (endothelial cells, Kupffer cells, fat-storing cells) occupy the margins of the sinusoids and are termed littoral cells.

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SEM of cryofractured pork liver 3 hr after slaughter showed hepatocyte chords separated by sinusoids (Fig. 2a). Because the liver was not perfused, sinusoids contained elements of the liver circulation. In the TEM image (Fig. 2b), the red blood cell marked the lumen of a sinusoid. Microvilli formed the sinusoidal face of each hepatocyte and the bile canaliculi between contiguous hepatocytes. Collagen in the Disse's space was not abundant, visible usually as cross sections of single fibers or small bundles. Ultrastructural changes had occurred in the tissue during the 3 hr postmortem period before fixation, e.g., mitochondria were clumped together and hepatocyte nuclei exhibited margination of chromatin. However, the tissue was representative of liver as a food and served as a control for these experiments.

After one cycle F-T, major changes were observed in sectioned tissue (Fig. 3a). Littoral cells were fragmented and microvilli of the Disse's space were gone. Sinusoids contained particulates and vesiculated membrane fragments. Glycogen appeared both inside hepatocytes and in sinusoids, and breaks in hepatocyte plasma membranes were visible. Rough endoplasmic reticulum was slightly dilated and smooth endoplasmic reticulum was not apparent. Some mitochondria were ghosts, while others retained normal volume and recognizable cristae. Condensation bodies in even the well-preserved mitochondria indicated profound damage. Nuclei and peroxisomes were compacted. Microvilli within bile canaliculi were partially destroyed but adhesion of hepatocytes along intercellular boundaries was maintained. SEM of this tissue (Fig. 3b) showed enlargement of sinusoids relative to hepatocytes. The fracture profiles of nuclei differed from the control (Fig. 2b), evidence of changes in nuclear membrane and matrix properties as a result of freezing.

Four and six cycle F-T samples were alike in appearance; only examples of four cycle F-T liver are presented (Fig. 4a). After four cycles F-T, remnants of hepatocytes could be identified as clumps of membranes and cell organelles. Rough endoplasmic reticulum, golgi and mitochondria were swollen but intact, whereas other cell membranes had vesiculated or formed multilamellar structures. Nuclei were generally absent, although a possible remnant of a nucleus appears at the top of Fig. 4a. Peroxisomes were not apparent. A few collagen fibers were still discernable at hepatocyte borders (not shown). Sinusoids contained cell ground substance, glycogen, and membrane vesicles, including mitochondrial ghosts. In general, four cycle F-T tissue in TEM images presented a rather homogeneous appearance. In SEM (Fig. 4b), however, hepatocyte-sinusoid borders could easily be distinguished. The SEM image, formed by differences in topography, is enhanced by slight differential tissue shrinkage that occurs during critical-point drying. It was apparent from topography of these fracture surfaces that hepatocyte remnants contained more mass than sinusoids, even though some redistribution of mass into sinusoids was evident from TEM images.

The results with liver refrigerated for up to six days at +5°C (Fig. 5) indicated that overall tissue organization was better maintained than in F-T samples, although cell deterioration occurred. After one cycle R-S (Fig. 5a), glycogen had spread to the sinusoids through breaks in hepatocyte plasma membranes. Cisternae of rough endoplasmic reticulum were swollen. Mitochondria were damaged but only slightly swollen. Remnants of littoral cells sometimes remained (not shown), but microvilli were gone. Collagen was not seen. Four and six cycle R-S tissues were alike; a micrograph of six cycle is presented in Fig. 5b. Hepatocyte cell boundaries were still well-defined and sinusoids contained less debris than in four cycle F-T (Fig. 4a). Nuclei were intact, mitochondria retained their content, and bile canaliculi were recognizable (not shown). Cell ground substance was flocculated and glycogen was scant, but there was no cytomembrane vesiculation as observed in four cycle F-T.

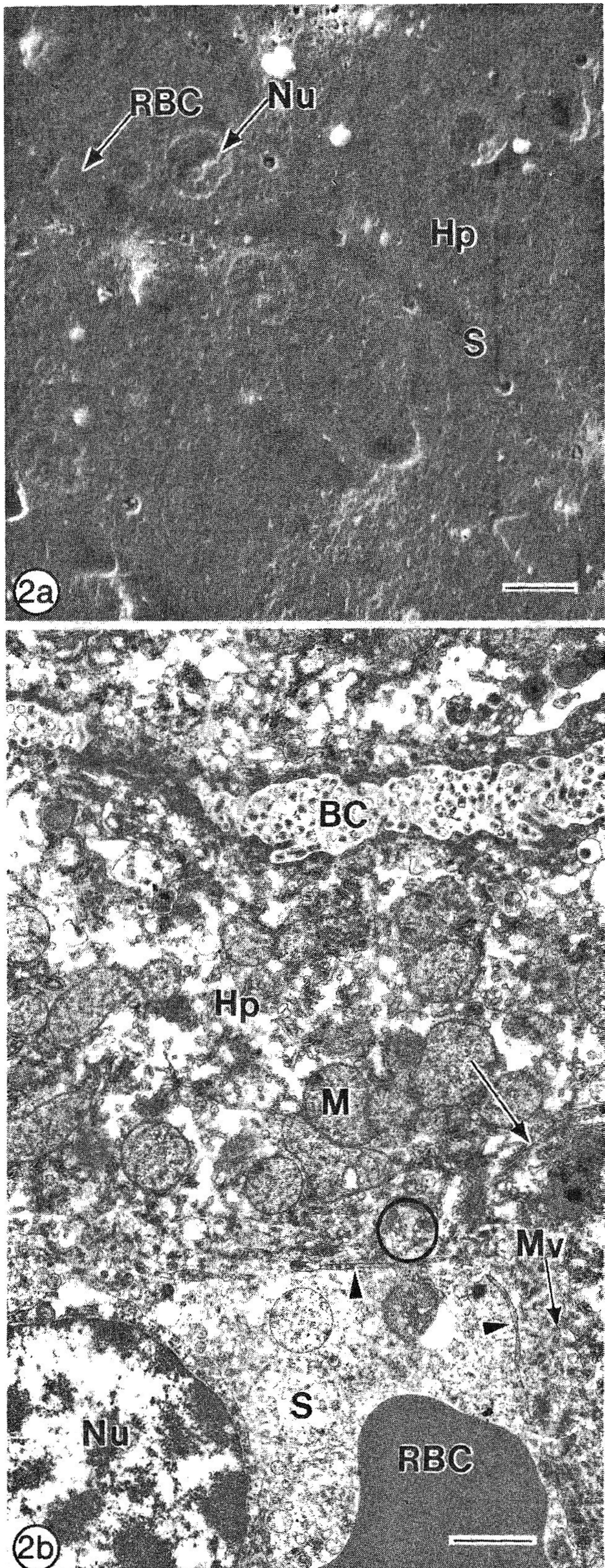

Fig. 2—Pork liver parenchyma fixed 3 hr after slaughter. 2a—Scanning electron micrograph (SEM) showing hepatocytes (Hp) separated by sinusoids (S). (2,000×, bar = 5 μm; scale applies to all SEMs). Nu = nucleus; RBC = red blood cell. 2b—Transmission electron micrograph (TEM) of a hepatocyte (Hp) bordering a sinusoid (S). Processes of littoral cells (arrowheads) marked the boundary of the sinusoid lumen. Collagen (white dots in circled area) was found in the Disse's space, between littoral cells and hepatocyte. (11,000×, bar = 1 μm; scale applies to all TEMs). BC = bile canaliculus; M = mitochondrion; Mv = microvilli; Nu = nucleus; RBC = red blood cell.

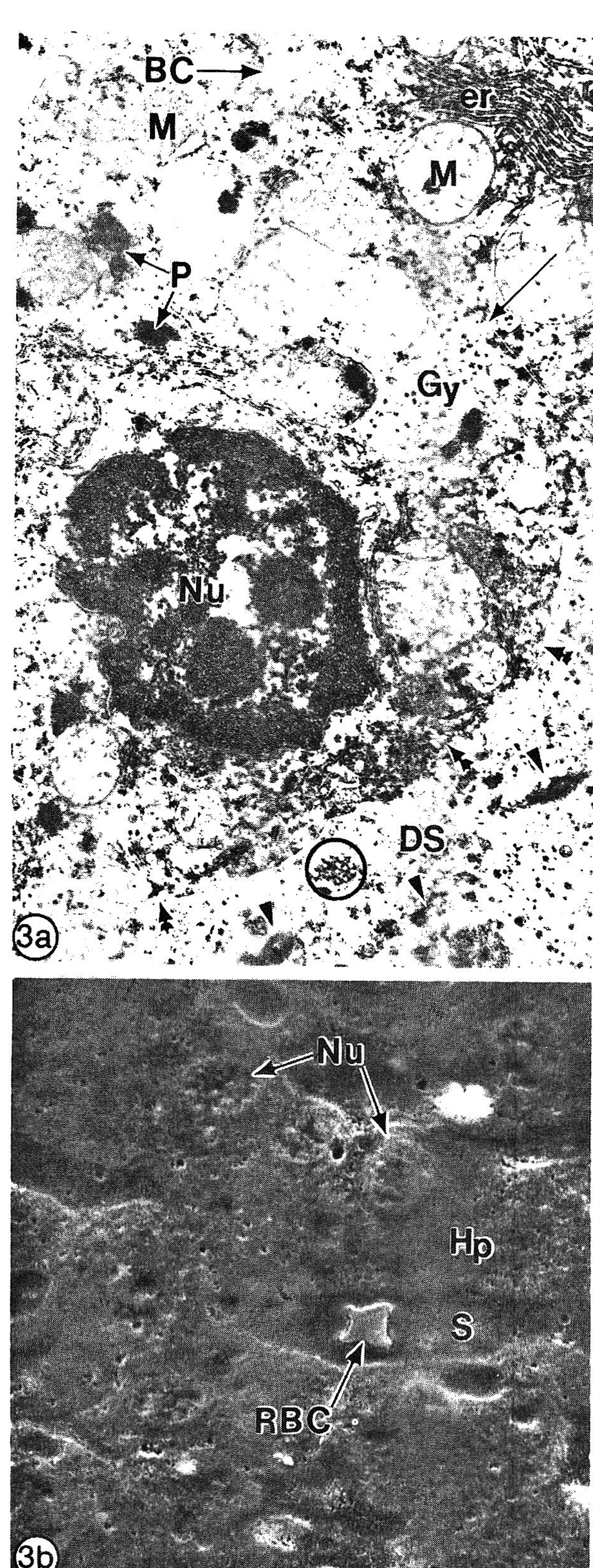

Fig 3—Pork liver frozen at − 20°C for 70 hr and thawed at + 5°C for 24 hr (1-cycle F-T). 3a—Remnants of littoral cells (single arrowheads) delimited an enlarged Disse's space (DS) which contained some collagen (circled). Microvilli were absent from the sinusoidal face of hepatocytes (double arrowheads) and reduced in number in bile canaliculi (BC). Intercellular adhesion of hepatocytes (single arrow) was not visibly altered (11,000×), Gy = glycogen; M = mitochondrion; Nu = nucleus; P = peroxisome; er = rough endoplasmic reticulum. 3b—SEM fracture surface showed hepatocyte (Hp) chords well defined by enlarged sinusoidal spaces (S) (2,000×), Nu = nucleus; RBC = red blood cell.

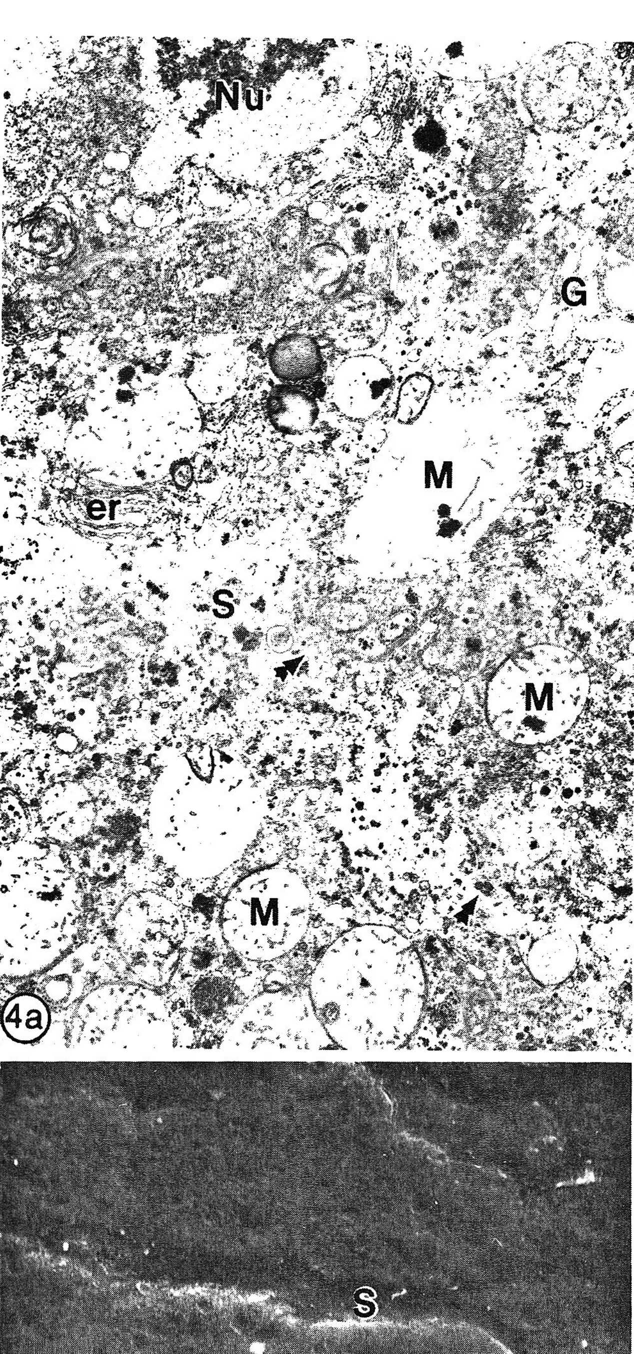

Fig. 4—Pork liver after four cycles of freeze-thaw. 4a—Boundaries of hepatocytes (double arrowheads) were difficult to locate in TEM images. Rough endoplasmic reticulum (er), golgi (G), and most mitochondria (M) were swollen but intact. Other cell membranes were converted to vesicles or multilamellar structures (11,000×). Nu = nucleus; S = sinusoid. 4b—SEM showed compaction of the tissue overall (2,000×), HP = hepatocyte; S = sinusoid.

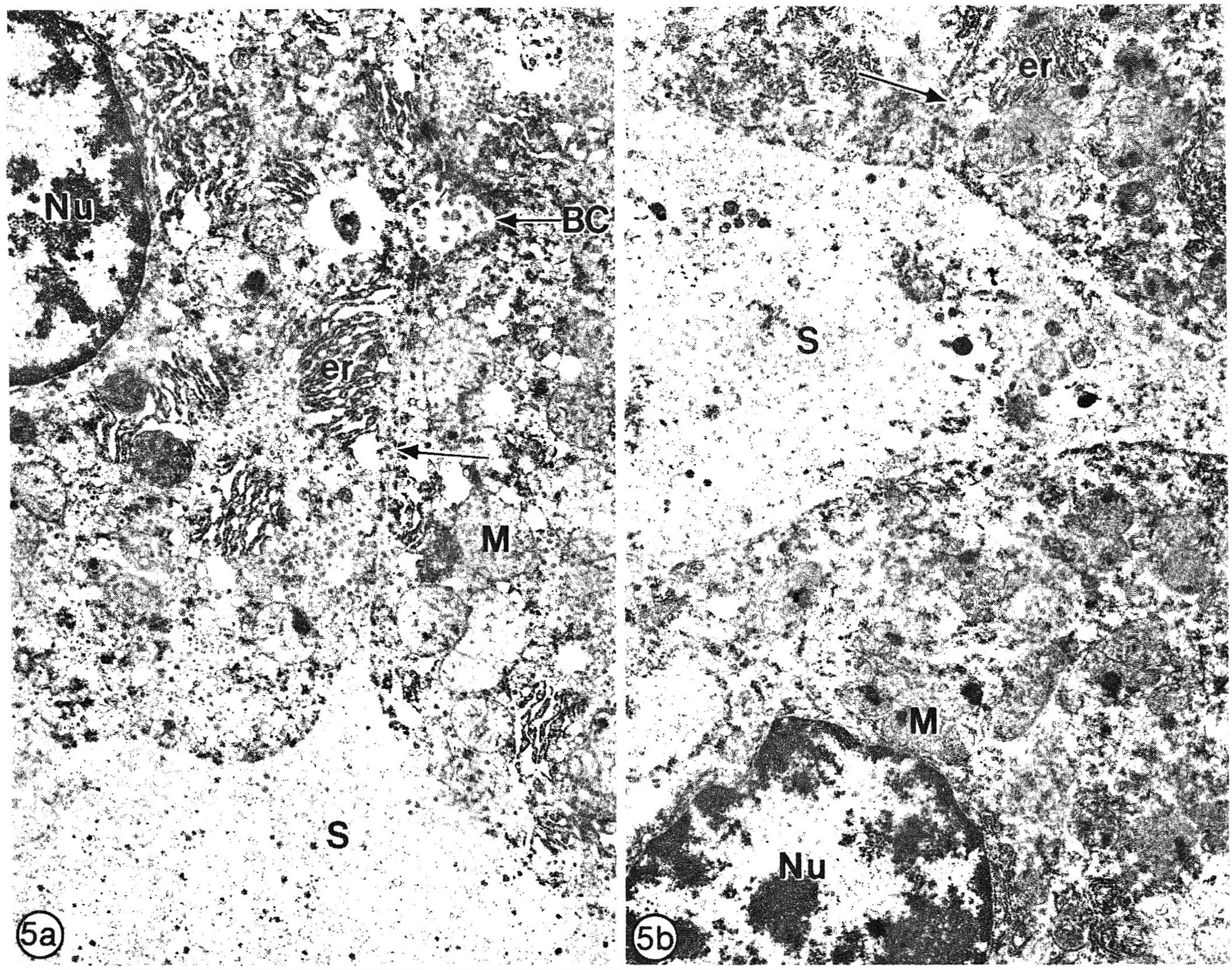

Fig. 5—Pork liver after refrigerated storage. 5a—After one day at +5°C (one cycle R-S), rough endoplasmic reticulum (er) was dilated and mitochondria (M) were slightly swollen. Nuclei (Nu) were intact and intercellular boundaries (arrow) were well preserved (11,000×), BC = bile canaliculus; S = sinusoid. 5b—After six days at +5°C (six cycle R-S), hepatocyte boundaries, both luminal and intercellular (arrow), were well defined. Sinusoids (S) contained less debris than did 4-cycle frozen-thawed specimens (11,000×), M = mitochondrion; Nu = nucleus, er = rough endoplasmic reticulum.

DISCUSSION

THE OVERALL HEPATOCYTE-SINUSOID organization of pork liver was stable during six days of refrigerated storage, although deterioration of hepatocytes was evident. Swelling of mitochrondria and rough endoplasmic reticulum indicated loss of ionic compartmentation, but these and other cell organelles persisted reasonably well during the time period.

Liver cells deteriorated more with freezing and thawing than with refrigerated storage. Glycogen, which served as a marker for migration of cytoplasmic constituents, was observed in sinusoids after both one cycle R-S and one cycle F-T, indicating that breakage of plasma membranes had occurred on the sinusoidal face of hepatocytes in both tissues. The extent of glycogen migration, however, was greater in F-T than in R-S tissue.

A specific effect of freezing was vesiculation of hepatocyte membranes. Nuclear membranes, smooth endoplasmic reticulum and microvilli appeared to be very susceptible to freeze-thaw damage, whereas rough endoplasmic reticulum, mitochondrial outer membranes, and plasma membranes along interhepatocyte borders remained in place. Mitochondrial damage after freezing was shown by swelling, loss of content, and the presence of condensation bodies. Hamm and Masic (1975) were able to distinguish frozen-thawed from fresh pork liver by the activity of mitochondrial aspartate aminotransferase (GOT) in hepatocyte cytoplasm. Even 4 days postmortem, freezing and thawing of liver produced nearly a doubling of the total GOT specific activity in the cytoplasm. Interestingly, in micrographs we observed two classes of mitochondria in roughly equal numbers. One class swelled and lost content; the other class maintained approximately normal appearance through at least four cycles F-T.

During slow freezing, a relative term that applies to conditions employed in this study, ice nucleation in tissue occurs in the extracellular compartment, producing dehydration of cells and concentration of their solutes (Franks et al., 1983). When slow thawing occurs, new ice crystals form by recrystallization (Toledo-Pereyra, 1982). The net effect of freeze-thaw is movement of bulk water within the tissue.

Evidence from micrographs showed that repetitive F-T caused displacement of the cellular fluid phase into the sinusoids, eventually resulting in compaction of remaining hepatocyte organelles and accumulation of soluble cellular components, small cytoplasmic particulates, and some cellular organelles in the sinusoids. Retention of this fluid within the sinusoids would explain the presence of a viscous exudate when F-T tissue was sliced. The results suggested that drips from F-T liver and R-S liver arise from different types of tissue damage. If composition of drip is closely related to sinusoid content, then drips

from F-T and R-S liver probably differ in gross composition. Drip from F-T liver may contain a higher proportion of cellular proteins and debris, while R-S liver drip may contain a higher proportion of blood components.

In conclusion, different types of liver tissue damage resulted from refrigerated storage vs. freeze-thaw cycling. Autolytic changes predominated in refrigerated liver, while freeze-thaw caused direct membrane fragmentation, movement of water, solutes and small particulates in the tissue, and compaction of residual cell organelles. Freeze-thaw damage to liver tissue was more severe than damage during refrigerated storage when structural changes alone were considered.

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are presented in Table 5. Few consistent differences were observed among treatments. The data indicate that under the storage conditions used in this study, chops would be considered desirable for at least 2 days of retail display after 28 days of storage. This conclusion is consistent with microbial counts (Table 1) and the other retail display characteristics (Table 5).

The results of this study indicate that rapid chilling of hot-boned pork had an advantageous effect on pork color; however, disadvantages included more shrinkage and increased Warner-Bratzler shear force values. These results indicate that hot processing and rapid chilling do not have beneficial effects on the shelf life and palatability attributes of fresh pork. However, the use of rapid chilling may be an economically important alternative for processing pork.

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Influence of Different Chilling Conditions on Hot-Boned Pork

K. O. HONIKEL and J. O. REAGAN

ABSTRACT

The objective of this study was to determine whether cold shortening affects porcine muscles with the intention of evaluating optimum chilling conditions for hot-boned pork. Fast chilling of the porcine *M. longissimus* below 10°C increased purge with little effect on toughness. The fast fall of pH induced by electrical stimulation at prevailing body temperatures has been associated with PSE characteristics. Rapid chilling may reduce or prevent this effect. Electrical stimulated/cold-boned loin segments exhibited higher levels of purge than those which were electrical stimulated/hot-boned and chilled rapidly. Cooking losses were not effected by hot-boning or electrical stimulation.

INTRODUCTION

HOT-BONING of carcasses has experienced a revival in the last few years for various reasons which are primarily economical. The current increase in energy costs and the possibility of producing meat with improved quality (Schmidt and Gilbert, 1970), lower costs and higher yield (Anon., 1980, 1983) by hot-boning has encouraged meat scientists to renew research in this area and evaluate optimal processing conditions for hot-boning by applying modern meat technology.

Several problems have been associated with hot-boning. The major problem is a decrease in meat tenderness due to muscle shortening of fast chilled excised cuts (Locker, 1960; Smith et al., 1976), along with an associated change in the shape of cuts. Furthermore, hot-boning followed by very fast or very slow chilling conditions prior to the onset of rigor mortis can lead to an increase in drip and weight loss in the cuts (Honikel et al., 1981).

Most of the studies on hot-boning have been carried out with beef and lamb. Locker and Hagyard (1963), Powell (1978) and Honikel et al. (1981) demonstrated that excised prerigor beef muscles conditioned at 12–19°C showed minimum shortening of muscle fibers and minimum drip loss.

Pork carcasses, however, differ greatly from beef and lamb in that postmortem breakdown of energy-rich compounds occurs in pork much faster than in beef (Honikel et al., 1984), resulting in an earlier onset of rigor mortis. Despite the faster glycolysis of porcine muscles, cold shortening has been observed in pork (Marsh et al., 1972; Fischer et al., 1980). Reagan (1983) reported higher purge values in brine-chilled hot-boned pork. Wynne (1980) observed few differences between hot-boned pork conditioned at 17°C for several hours and conventionally cold-boned, vacuum packaged pork.

If hot-boning of pork is to become an efficient and feasible alternative in the meat industry, optimal chilling time and temperature conditions must be determined. Under practical conditions in a slaughter plant, however, biochemical and temperature changes occur simultaneously. Decreasing temperature is accompanied by decreasing pH.

The objectives of this study were to determine the effects of chilling time and temperature upon the biochemical changes and meat quality characteristics of hot-boned pork.

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MATERIALS & METHODS

NINE MARKET WEIGHT HOGS (80–100 kg) were slaughtered using conventional methods and skinned. In addition three hogs were slaughtered as above and within 10 min postmortem were electrically stimulated (stimulation time 30 sec, 550 V with 2 sec power on, 1 sec power off). The carcasses were then split in half. The right half served as control and was chilled for 24 hr at 10°C. From the left side of the carcass the *M. longissimus dorsi* was excised and trimmed of subcutaneous fat. Within 1 hr postmortem the muscles were cut into three equal segments (weighing 550–850g each) and assigned to three different temperature treatments. The sirloin end of the loan of nonstimulated carcasses was designated T1, the middle part T2 and the blade end T3. The corresponding parts from the contro. side were designated C1, C2, and C3.

After cutting, all segments were put immediately in shrinkable barrier bags (W.R. Grace, Cryovac), but not sealed. Segment T1 and all stimulated and hot-boned samples (designated EST1, EST2 and EST3) were inserted into a water bath at 5°C (the mouth of the bag was above water level). Segment T2 was inserted in a water bath at 11°C and T3 in a water bath at 17°C. At various times postmortem the temperature, pH and R-value were determined. At 5 hr postmortem the hot-boned samples were vacuum packaged in shrinkable barrier bags (2 mm thick with an oxygen transmission rate of 30-40 $cm^3/cm^2/24$ hr at 23°C and a moisture vapor transmission rate of 1.0 $cm^3/cm^2/24$ hr at 38°C and 100% RH with a Multivac AG 500 with a vacuum setting of 5.5 (approximately 1.0 mm Hg). The vacuum packaged bags were heat shrunk (3 sec at 90°C water bath) and stored at −1°C for 20 hr.

The control sides were chilled for 24 hr postmortem at 10°C. Temperature, pH and R-values were measured in the M. longissimus dorsi in similar positions to those taken from the hot-boned segments. After 24 hr the M. longissimus dorsi was excised, vacuum packaged and heat shrunk in the same manner as the hot-boned segments and stored together with the hot-boned segments for another 6 days at −1°C, after which purge (exudate of vacuum packaged meat) was determined. All samples were repacked under vacuum and stored in a −20°C freezer. Samples were later thawed in a 5°C cooler for 24 hr prior to cooking loss and shear force determinations.

pH

The pH values of the muscles were determined using a Corning Model 610 A portable pH-meter with an Ingold Model 6:6 puncture type electrode.

R-Value

The determination of R-value (Honikel and Fischer, 1977) is a fast spectrophotometric method for the estimation of the degree of transformation of ATP to IMP and serves, therefore, as an indicator of ATP breakdown in postmortem muscle (Jolley et al., 1980/81).

Temperature

Temperature was measured using CYSI, Model 42SC Telethermometer with a No. 418 probe.

Shear force determination

Shear force values were determined from cores (1.27 cm diameter) obtained from chops (2.54 cm thick) cooked to an internal temperature of 70°C. Peak shear force was measured for each core using an Instron Universal Testing Instrument (Model 1009) with a Warner-Bratzler shearing attachment. The mean of four values was taken for each muscle sample.

Cooking loss

Samples (weighing 90–130g) were broiled to an internal temperature of 70°C on Farberware open face broiler units (Model 450 N).

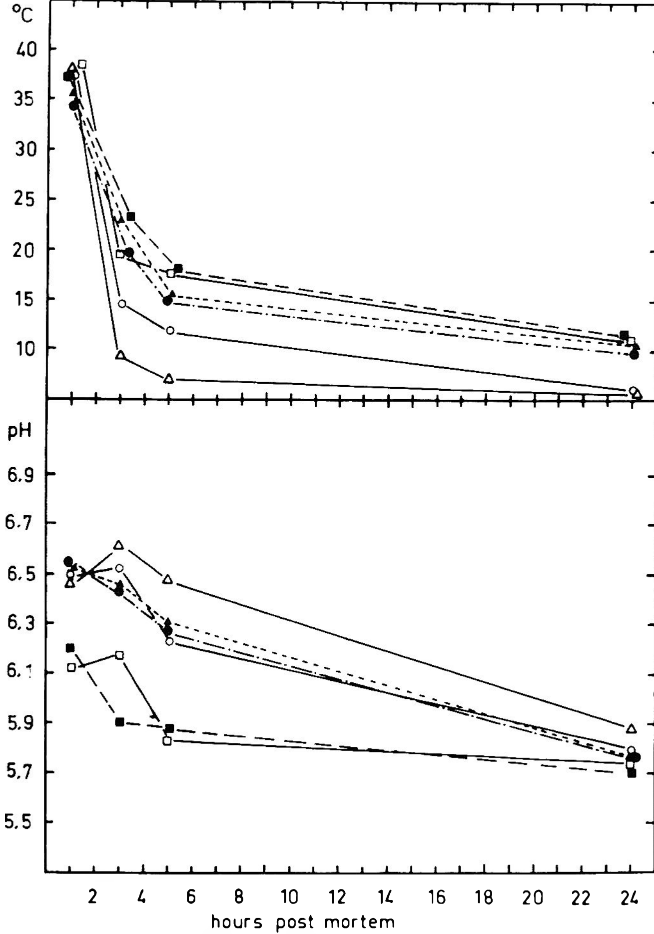

Fig. 1—Temperature and pH changes by time postmortem in three segments of M. longissimus dorsi hot-boned and chilled under different temperature conditions and within the carcass. Temperature and pH values are means of nine samples each: △—△ T 1; ▲—▲ C 1; ○—★ T 2; ●---● C 2; □—□ T 3; ■-—■ C 3; Chilling conditions: see Materials & Methods.

Values for percentage cook loss was determined from samples weighed prior to and after cooking.

Statistical methods

Students t-test was performed to determine significance between treatments and cuts. Significance differences were accepted at the 5% level of probability.

RESULTS & DISCUSSION

Influence of chilling conditions on temperature and pH decline

The three segments of the loin immersed in water baths at 5°, 11° and 17°C attained those temperatures in the center of each loin segment with 5 hr (Fig. 1). At the same time the temperatures from the loin in the carcass (cold-boned side) were measured at similar positions. In the hot-boned samples, temperatures dropped very quickly in the beginning, whereas in the control sides a slower temperature decline was observed. Temperature variations among the samples within the various treatment groups were small (Table 1). The mean values in pH declined with time, postmortem, and showed in nonstimulated samples relatively high standard deviations of about ± 0.2 pH units (Table 1). In the sirloin end and in the middle of the loin, pH dropped fairly slowly; however, pH decline in the blade end was rather rapid (Fig. 1). The rate of pH fall in hot-boned and the corresponding segments within the carcass was similar (Table 1). The lower pH values associated with the blade end segments (T3, C3) were evident prior to the incubation of these segments. This indicates that the rapid pH drop was not due to differences in incubation temperature, but due to different rates of glycolysis within the M. longissimus dorsi. This observation was also noted by Lundström (1984).

The increase in R-value corresponded to the pH changes. Segments T3 and C3 had the highest R-value (lowest ATP concentration) at 1 hr postmortem (Table 1).

In the electrically stimulated samples, pH values dropped rapidly within 1 hr postmortem to below 5.75 at corresponding temperatures of 35°C and higher (Table 1). The final pH was attained in these samples within 1–1.5 hr postmortem. Electrically stimulated hot-boned samples were stored in 5°C water bath and chilled rapidly, like segment T1. The control samples which remained in the electrically stimulated control sides, chilled slowly similarly to the nonstimulated control samples C1, C2 and C3.

Variable relationships existed between temperature decline and pH changes with time postmortem in the segments and between hot- and cold-boned samples. Different structural changes of myofibrillar proteins may have occured.

Influence of chilling conditions on purge, cooking loss and shear force

In segments T1, with their fast decrease of temperature below 10°C within 3 hr but high pH values at about 6.45, cold shortening could have taken place. In segment T2, with milder chilling conditions reaching 15°C and 12°C at 3 and 5 hr, respectively, pH-values between 6.35 and 6.25 were observed. Under these pH and temperature conditions no, or little, shortening should have occured according to Honikel et al. (1984). In segment T3 where the pH fall was rather rapid and the chilling was moderate no prerigor shortening was likely. In the control segments C1, C2 and C3 the chilling rates were comparable to those of segments T3 (Fig. 1). Cold shortening could not occur and considerable rigor shortening was unlikely as the muscle remained in the carcass.

Electrical stimulation with its fast pH fall at prevailing high temperatures above 35°C at 1 hr postmortem may lead to PSE-like conditions. Fast chilling of PSE muscles, however, reduced the negative quality effects on purge and color (Honikel et al., 1984). Therefore, all stimulated hot-boned samples, which were fast chilled in a 5°C water bath should relinquish a part of the PSE-like conditions. ES-cold-boned samples, however, should exert PSE-characteristics. The mean values for purge, cook loss and shear force determinations for the different loin segments are shown in Table 2. Percentage cook loss for all samples did not differ significantly among the segments and treatments despite large differences in pH fall between stimulated and nonstimulated samples. This is not surprising, as Honikel et al. (1980, 1984) showed in beef and pork, respectively, that cook loss, but not drip loss, was independent of shortening and conditioning temperature and rate of pH fall, but dependent on the pH of the samples. Since all of the samples had a final pH value between 5.6 and 5.7 these results would be expected.

Purge values, however, differed (Table 2). In nonstimulated samples the mean values range from 1.46 to 2.53%. The significant differences in these samples were observed between hot- and cold-boned segments not only in the corresponding T1 and C1, but also between segments T1 and T2 with T3 on comparison between treatments. The difference in purge between T1 and the other segments is most probably due to cold shortening of T1 as proposed earlier in this paper whereas in the other nonstimulated segments, either hot- or cold-boned, no shortening should have taken place and no significant differences in purge losses were observed.

In the segment pairs, the rapid chilling of the hot-boned samples reduced purge (Table 2). Due to the small number of samples with a wide variation of purge, however, no significant differences could be observed. As previously stated, fast

Table 1—Changes of temperature and biochemical parameters postmortem in three parts of M. longissimus dorsi of hogs treated at different temperatures[a]

Treatment		Temperature in muscles (°C)			pH			R-value	
Code[b]	Temp. of water bath °C	1	3 hr postmortem	5	1	3 hr postmortem	5	1 hr postmortem	5 hr postmortem
T 1	5	37.7 ± 1.2	9.0 ± 1.8	6.9 ± 0.5	6.5 ± 0.2	6.45 ± 0.3	6.2 ± 0.3	0.89 ± 0.07	1.02 ± 0.05
C 1		36.0 ± 1.9	23.1 ± 1.8	16.4 ± 2.2	6.55 ± 0.2	6.2 ± 0.3	6.1 ± 0.35	0.88 ± 0.05	1.01 ± 0.09
T 2	11	37.3 ± 1.4	14.8 ± 2.6	11.8 ± 1.2	6.45 ± 0.2	6.35 ± 0.2	6.25 ± 0.3	0.88 ± 0.05	0.99 ± 0.08
C 2		34.2 ± 2.7	19.9 ± 2.6	14.9 ± 2.6	6.5 ± 0.2	6.3 ± 0.4	6.2 ± 0.3	0.87 ± 0.02	1.01 ± 0.12
T 3	17	38.7 ± 1.2	19.3 ± 1.6	17.5 ± 0.6	6.0 ± 0.3	5.9 ± 0.35	5.8 ± 0.3	1.00 ± 0.13	1.12 ± 0.08
C 3		37.3 ± 1.3	23.8 ± 3.1	17.9 ± 3.1	6.2 ± 0.3	5.9 ± 0.3	5.75 ± 0.2	1.00 ± 0.06	1.16 ± 0.03
ES T 1	5	37.7 ± 1.3			5.6 ± 0.2				
ES C 1		37.5 ± 1.0			5.75 ± 0.1				
ES T 2	5	35.0 ± 0			5.75 ± 0.15				
ES C 2		35.2 ± 1.5			5.65 ± 0.15				
ES T 3	5	38.0 ± 0.9			5.35 ± 0.05				
ES C 3		39.2 ± 1.0			5.55 ± 0.02				

[a] in T 1 to C 3: mean value and s.d. of nine animals; ES T 1 - ES C 3: mean values and s.d. of three animals.

[b] Abbreviations of code: T: hot boned sample; C: cold boned sample. 1: sirloin end of M. longissimus dorsi; 2: center of M. longissimus dorsi; 3: blade end of M. longissimus dorsi; ES: electrically stimulated in carcass.

Table 2—Purge, cooking loss and shear force in loins assigned to different temperature treatments

Treatment[d]	Number of samples	% Purge $\bar{x}$ ± s	% Cooking loss $\bar{x}$ ± s	Shear force (kg) $\bar{x}$ ± s
T 1	9	2.36 ± 0.47[a]	23.7 ± 2.5	2.8 ± 0.55
C 1	9	1.70 ± 0.45[c,b]	23.7 ± 5.1	2.6 ± 0.60
T 2	9	1.65 ± 0.19	22.7 ± 5.0	2.8 ± 0.65
C 2	9	1.61 ± 0.21[c,d]	22.0 ± 5.8	2.75 ± 0.50
T 3	9	1.48 ± 0.35[b]	23.3 ± 5.0	2.4 ± 0.40
C 3	9	1.46 ± 0.02[c,d]	20.4 ± 3.9	2.15 ± 0.60
ES T1	3	1.78 ± 0.69[a,b,c]	21.0 ± 2.05	2.6 ± 0.45
ES C1	3	2.25 ± 0.80[a,b,c]	22.1 ± 2.7	2.65 ± 0.75
ES T2	3	1.73 ± 0.73[a,b,c]	23.6 ± 6.35	2.45 ± 0.35
ES C2	3	1.97 ± 0.33[a,b,c]	23.1 ± 6.6	2.5 ± 0.3
ES T3	3	1.83 ± 0.60[a,b,c]	18.7 ± 7.15	2.25 ± 0.55
ES C3	3	2.53 ± 1.12[a,b,c]	24.4 ± 4.58	2.25 ± 0.45

[a-c] Means possessing different subscripts are significantly different.

[d] Code-see Table 1

chilling of PSE-prone muscle reduced the negative quality characteristics of PSE-muscles expressed as their decreasing purge.

Shear force determinations (Table 2) showed great variation among segments of the same muscle, as well as variation between the corresponding hot-boned and cold-boned segments and were not significant (P>0.01). The structural differences of the three muscle segments seemed to be of greater influence than that of boning treatment. In the stimulated samples where hot- and cold-boned samples went into rigor prior to cutting these differences did not exist (Table 2).

CONCLUSIONS

HOT-BONING OF PORK carcasses followed by chilling did not influence the fall of pH at corresponding muscle segments. Purge increased in unstimulated carcasses chilled rapidly to temperatures below 10°C at pH values around 6.4, i.e. prior to the onset of rigor mortis. Slower chilling regimes did not result in differences in purge. Muscles in carcasses under conventional conditions did not experience such a rapid decline in temperature. Electrical stimulation, however, demanded fast chilling to avoid PSE-like conditions resulting from a rapid pH fall at high temperatures. Cooking loss remained basically unchanged and was independent of hot- or cold-boning, chilling rate or electrical stimulation. The shear force of cooked samples, which was in all cases quite low, tended to have lower values for cold-boned samples and hot-boned samples under slow chilling conditions. Differences between segments of the same muscle and between carcasses were larger than the other influencing parameters. Hot-boning of hog carcasses is therefore a feasable procedure as has been previously shown for beef.

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Effect of Cathepsin D on Bovine Myofibrils under Different Conditions of pH and Temperature

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ABSTRACT

Purified cathepsin D was incubated with bovine skeletal muscle myofibrils under *in vitro* conditions resembling those found in postmortem muscle. SDS-PAGE analysis of myofibrils treated at pH 5.5 and 37° C and the sedimented, showed degradation of myosin heavy chains and titin. A small amount of actin, tropomyosin, troponins T and I, and myosin light chains also were degraded. The cathepsin D treated myofibrils were not fragmented to any greater extend than untreated myofibrils. Raising the pH and/or lowering the temperature greatly reduced the effectiveness of cathepsin D suggesting that the enzyme does not play a principal role in the tenderization process occurring in muscle postmortem.

INTRODUCTION

CHANGES in meat texture that occur postmortem are most likely due to the action of endogenous proteolytic enzymes (Bird and Carter, 1980; Goll et al., 1983). Cathepsin D is an endogenous enzyme, which has been proposed to participate in this process (Dutson, 1983). The increase in meat tenderness that accompanies postmortem aging is assumed by many investigators to be the result of breaking, fragmenting or at least weakening of the myofibrillar structure at or near the Z-line (Parrish et al., 1973; Lawrie, 1980, 1983; Robson et al., 1980, 1981, 1984; Goll et al., 1983). Therefore, the action of an enzyme that is effective in postmortem muscle should be to catalyze the hydrolysis of one or more proteins at a structurally important site that results in weakening of the myofibril. Although a number of studies have been done to assess the relative effectiveness of cathepsin D in its ability to hydrolyze myofibrillar protein, few studies have taken into account the chemical and physical environment that is present in postmortem muscle.

Cathepsin D is a carboxyl protease, and its molecular weight in different tissues has been reported over a range of 42,000 to 53,000 daltons (Barrett and Heath, 1977; Lah and Turk, 1982; Samarel et al., 1984). Cathepsin D isolated from bovine or human muscle has a reported molecular weight of about 42,000 daltons, but also exhibits two bands corresponding to proteins of about 28,000 and 14,000 daltons by SDS-PAGE (Barrett and Heath, 1977). However, the enzyme isolated from rabbit cardiac muscle has been reported to contain a 48,000-dalton chain derived from a 53,000-dalton precursor (Samarel et al., 1984). Native cathepsin D exists as multiple forms when examined by isoelectric focusing, with pI's of the isomers falling in the range 5–6. The enzyme purified from rat skeletal or cardiac muscle, for instance, contained three major forms on isoelectric focusing gels (Barrett and Heath, 1977, Samaral et al., 1984).

It has been shown that cathepsin D maximally degraded purified native myosin and actin at a pH of 4.0 (Schwartz and Bird, 1977). Myosin degraded by cathepsin D activity initially showed heavy-chain fragments in the 175,000- to 150,000-dalton region. These large fragments were then further degraded to many fragments of molecular weight less than 100,000 daltons (Schwartz and Bird, 1977). Each of three isoelectric focusing isomers of cathepsin D produced the same reaction products when incubated with myosin and actin (Schwartz and Bird, 1977).

Schwartz and Bird (1977) also found that cathepsin D degraded F-actin optimally at pH 4.0, however the rate of actin hydrolysis was only about 10% of that observed with myosin. In general, it was concluded that cathepsin D was more effective in degrading myosin and actin than was cathepsin B (Schwartz and Bird, 1977). Other studies (Ogunro et al., 1979) have shown that cathepsin D isolated from cardiac muscle had activities toward myosin and actin similar to those of the skeletal muscle enzyme except that the light chains of myosin also were degraded by the cardiac enzyme. Recently, it was shown that addition of cathepsin D to purified muscle proteins resulted in degradation of myosin heavy chains, tropomyosin, troponin T, troponin I, and alpha-actinin, but not of actin (Matsumoto et al., 1983). It also has been reported (Scott and Pearson, 1978) that cathepsin D degraded soluble collagen, or its cross-linked peptides, to a limited degree.

The goal of this study was to examine the effect of purified cathepsin D on myofibrillar proteins and structure under postmortem-like conditions of pH and temperature and, in addition, to gain a greater understanding of cathepsin D's potential participation in the myofibrillar disassembly process postmortem.

MATERIALS & METHODS

Myofibril preparation

Myofibrils were prepared from bovine *longissimus* muscle. Approximately 100g muscle was removed within 1 hr after exsanguination. The muscle was trimmed free of excess fat and connective tissue and passed through a prechilled meat grinder. Myofibrils were prepared from 50 g ground muscle according to the method of Goll et al. (1974).

Cathepsin D preparation

Cathepsin D was extracted from bovine hearts within 1 hr after exsanguination according to the method of Barrett and Heath (1977), with the following modifications for cardiac muscle. The muscle was carefully trimmed free of fat and connective tissue. Only the left ventricle of each heart was used for the preparation. The trimmed muscle was ground through a prechilled meat grinder, and 2.5 kg of the ground tissue was homogenized in a 4 1-capacity Waring Blendor in 2.5 volumes of 0.15M NaCl, 1 mM EDTA, 2% n-butanol, pH 7.2. The extraction procedure yielded approximately 2g crude cathepsin preparation.

Cathepsin D was subsequently purified by a two-step affinity chromatography procedure similar to that used by Afting and Becker (1981). The crude enzyme was first chromatographed on concanavalin A-Sepharose, which separated cathepsin D and other glycoproteins from the mixture. Cathepsin D was eluted from concanavalin A-Sepharose by using α methyl mannoside and affinity-chromatographed a second time utilizing the peptide inhibitor of the enzyme (pepstatin) covalently bound to the Sepharose matrix. Cathepsin D was eluted from

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this column by using a pH gradient to dissociate the enzyme. After chromatography, the active fraction was dialyzed vs. 2 mM sodium phosphate, pH 6.5, and stored at 0–4°C.

Cathepsin D assay with hemoglobin

Because cathepsin D readily hydrolyzes denatured hemoglobin at pH 3.0 to 4.0 (Canonico and Bird, 1970), a hemoglobin assay was used as one of the measures for determining cathepsin D activity. An 8% (w/v) solution of bovine hemoglobin was dialyzed against water for 2 to 4 days and then centrifuged at 100,000 × g_{max} for 30 min. Steps in the assay were as follows: 0.25 mL 8% hemoglobin plus 0.25 mL 1.0M Na formate, pH 3.8, were preincubated at 37° C for 10 min, then 0.5 mL enzyme added, and the reaction mixture incubated for 1 hr at 37° C. The reaction was terminated by the addition of 3.0 mL 5% trichloroacetic acid (TCA) and the tubes were centrifuged for 10 min at 1,000 × g_{max}. The amount of activity was determined by using the modified Folin-Lowry (Lowry et al., 1951) procedure for determining quantity of TCA-soluble peptides in the supernatants as described by Barrett and Heath (1977). The specific activity of cathepsin D was expressed as mg tyrosine equivalent/mg enzyme/60 min at 37° C. Confirmation that activity was due to cathepsin D rather than to another contaminating protease was done in two ways. First, controls containing 40 μL 0.01M pepstatin in the hemoglobin assay tubes were used, demonstrating that hemoglobin hydrolyzing activity was inhibited by pepstatin, an effective inhibitor of cathepsin D. Second, the enzyme preparation also was assayed for the ability to hydrolyze N-benzoyl-DL-arginine β naphthylamide (BANA) or benzyloxycarbonyl-arginylarginine 2-naphthylamide (Z-arg-arg) (Sigma Chemical Co.) (Barrett and Heath, 1977) which would indicate the presence of other catheptic enzymes such as cathepsins B, H, or L.

Cathepsin D assay with myofibrils

The myofibrils were prepared from bovine longissimus muscle and assayed under the following conditions: 2.0 mg/mL myofibrils, 100 mM KCl, 50 mM imidazole-HCl of desired pH, 1 mM EDTA, plus enzyme. The enzyme to substrate ratio was kept at 1:100 (w/w). The pH values tested were 7.5, 6.5, and 5.5. At each of the pH values, temperatures of 37°, 25°, and 15° C were tested. The following two controls were included: (1) myofibrils plus pepstatin-no enzyme and (2) myofibrils plus pepstatin plus enzyme. The tubes were preincubated for 10 min at the desired pH and temperature. The reactions are initiated by addition of enzyme or an equal volume of solution without enzyme (for myofibrils plus pepstatin-no enzyme control tubes). The reactions were run for 30, 60, 90, and 120 min, then terminated by addition of 20 μL 0.01M pepstatin. The reaction tubes were centrifuged at 50,000 × g_{max} for 15 min at the temperature used in the reaction incubation. The proteolytic activity was determined at each time point by measuring the amount of protein in the supernatant by the Folin-Lowry method.

Determining myofibril fragmentation and conditions for polyacrylamide gel electrophoresis

Myofibril fragmentation and polyacrylamide gel electrophoresis in denaturing and nondenaturing buffers was done as described in the accompanying paper (Zeece et al., 1986).

RESULTS & DISCUSSION

PURIFIED CATHEPSIN D had a specific activity of approximately 240 units/mg by the hemoglobin assay with a typical yield of 3–4 mg from 2.5 kg of cardiac muscle. The enzyme preparation had no activity against Z-arg-arg and BANA, which indicated the absence of other catheptic enzymes.

When the purified cathepsin D was electrophoresed on a 10% polyacrylamide gel in a nondenaturing buffer system at pH 7.5 as shown in Fig. 1a, the gel contained three closely spaced bands of approximately equal intensity. When the cathepsin D was subjected to SDS-PAGE on a 10% polyacrylamide slab gel, two bands were observed (Fig. 1b) with mobilities corresponding to molecular weights of about 47,000 daltons for the major band and 29,500 daltons for the minor band.

Two-dimensional electrophoresis (nondenaturing at pH 7.5, followed by SDS-PAGE) has been utilized to examine the subunit composition of the three bands observed on nondenaturing gels (results not shown). It was found that the two fastest-

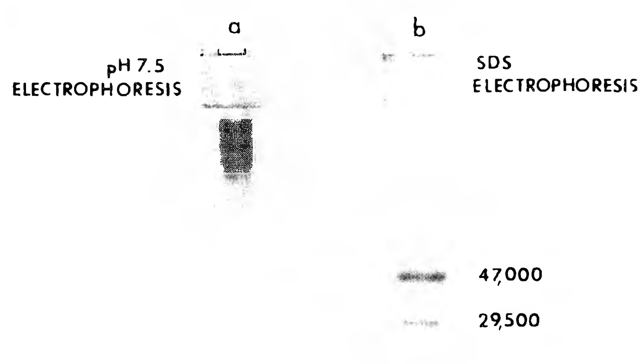

Fig. 1—Polyacrylamide gel electrophoretic examination of purified cathepsin D under nondenaturing and denaturing conditions. Purified cathepsin D (16 μg) was electrophoresed under nondenaturing conditions at pH 7.5 on a 10% acrylamide gel (a). Purified cathepsin D (13.8 μg) also was electrophoresed under denaturing conditions in the presence of SDS on 10% acrylamide gel (b).

fastest-migrating bands in the nondenaturing gel (Fig. 1a) contained only the 47,000 dalton component, while the slowest migrating band contained both 47,00- and 29,500-dalton components.

There has been little consensus about the native molecular weight of cathepsin D and its subunit composition. One group of investigators has reported that cathepsin D isolated from porcine spleen contained three to five isozymes with native molecular weights of approximately 50,000 daltons (Huang et al., 1979). Other investigators have reported the presence of a 100,000 dalton native species, which they suggested was a proenzyme form of cathepsin D (Puizdare and Turk, 1981). Ogunro et al. (1979, 1982) found that cathepsin D purified from canine cardiac muscle had a native molecular weight of approximately 50,000 daltons as measured by gel filtration. Canine cardiac cathepsin D contained two subunits with molecular weights of approximately 32,000- and 14,000 daltons (Ogunro et al., 1980; Samarel et al., 1981) by SDS-PAGE.

Clearly, direct comparisons and reconciliation of the electrophoretic results presented here on cathepsin D with those of other investigators is difficult. This may partly result from differences in SDS-PAGE systems used. More recently Sameral et al., 1984, have shown rat cardiac cathepsin D to contain a 48,000 dalton chain component, which is derived from a 53,000 dalton precursor. These investigators, as well as Barrett and Heath (1977), also reported the presence of at least three isomers of the 48,000 dalton component, which is in close agreement with results shown in Fig. 1.

Purified cathepsin D's ability to cause the release of soluble protein during incubation of myofibril suspensions was used as one measure of the enzyme's activity under different conditions of pH and temperature. Figure 2 shows the release of soluble protein after incubation with cathepsin D at pH 5.5 and 37°, 25°, or 15° C. A significant amount of protein was solubilized by cathepsin D after 60 min at 37°C. However, cathepsin D solubilized substantially less protein after 120 min at 25° C than after 120 min at 37° C. When incubations were conducted at 15° C, little or no increase in soluble protein was observed (Fig. 2).

When myofibrils were incubated with cathepsin D at pH 6.5 or 7.5 (at 37, 25, or 15°C), little or no release of soluble protein was observed except for a small increase for the incubation at pH 6.5 and 37° C (results not shown). In general, cathepsin D's ability to cause the release of soluble protein was very temperature dependent, and SDS-PAGE analysis of the soluble fraction has shown it to be composed primarily of low-molec-

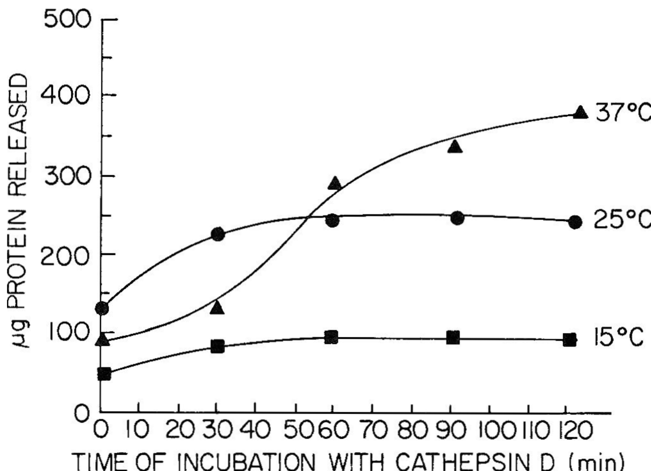

Fig. 2—Protein released from myofibrils after incubation with purified cathepsin D at pH 5.5. Bovine skeletal myofibrils were incubated with purified cathepsin D. Each sample was then sedimented at 50,000 × gmax for 15 min at the temperature of incubation. Protein remaining in the supernatant was measured by the Folin-Lowry method. Incubations were conducted at pH 5.5 and 37° C (▲—▲), 25° C (●—●), or 15° C (■—■).

ular-weight components (Fig. 3). The major products of hydrolysis (fragments of myosin) were obviously not soluble.

The ability of cathepsin D to hydrolyze myofibrillar proteins under different conditions of pH and temperature was investigated by SDS-PAGE (Fig. 3-5). Myofibrils were incubated with cathepsin D, centrifuged to separate supernatant and sediment fractions, and then examined by electrophoresis. Figure 3 shows the results obtained on supernatants and sediments from myobrils incubated with cathepsin D at 37° C and pH 5.5. The controls included one tube with myofibrils plus pepstatin and no enzyme, and another tube with myofibrils plus pepstatin, plus enzyme. Examination of the supernatant lanes (a-f) shows that very little protein was solubilized by the cathepsin D except for some small-molecular-weight material that traveled with the tracking dye at the bottom of the gel (lanes c-f). This suggests that the increase observed in protein released under these conditions (Fig. 2) was due primarily to release of very low-molecular-weight material. Some intact tropomyosin subunits were released, presumably by the low pH treatment, into all of the supernatant samples, including the controls. In the sediment lanes (g-l), even the two controls (lanes g and h) contained little titin, and that small amount was decreased further during the incubation. The decreased amount of titin observed in the controls under these conditions may be due to decreased solubility in SDS after low pH treatment. After 30 min of incubation (lane i), a great deal of degradation of myosin heavy chains had occurred, and new bands were observed at about 155,000 and 90,000 daltons. The pattern of myosin heavy-chain degradation seen in Fig. 3 was similar to that observed by Schwartz and Bird (1977). Those investigators reported that cathepsin D treatment of myosin produced myosin heavy-chain fragments of 150,000 to 175,000 daltons and of 110,000 daltons. There was a slight decrease in the actin band with increasing incubation time. Troponin T isomers were somewhat degraded, and tropomyosin was only slightly decreased in amount. Four to six faint bands appeared in the region of the gel below tropomyosin after 90 (lane k) to 120 min (lane l) of incubation. Troponin I and the strong band just above it (myosin light chain 1) also were partly degraded with increasing incubation time. Similar results were previously obtained by using rabbit skeletal muscle cathepsin D treatment of rabbit skeletal myofibrils by Matsumoto et al., (1983).

Figure 4 contains the electrophoretic results obtained on supernatants and sediments from myofibrils incubated with cathepsin D at 25° C and pH 5.5. Very little protein was released

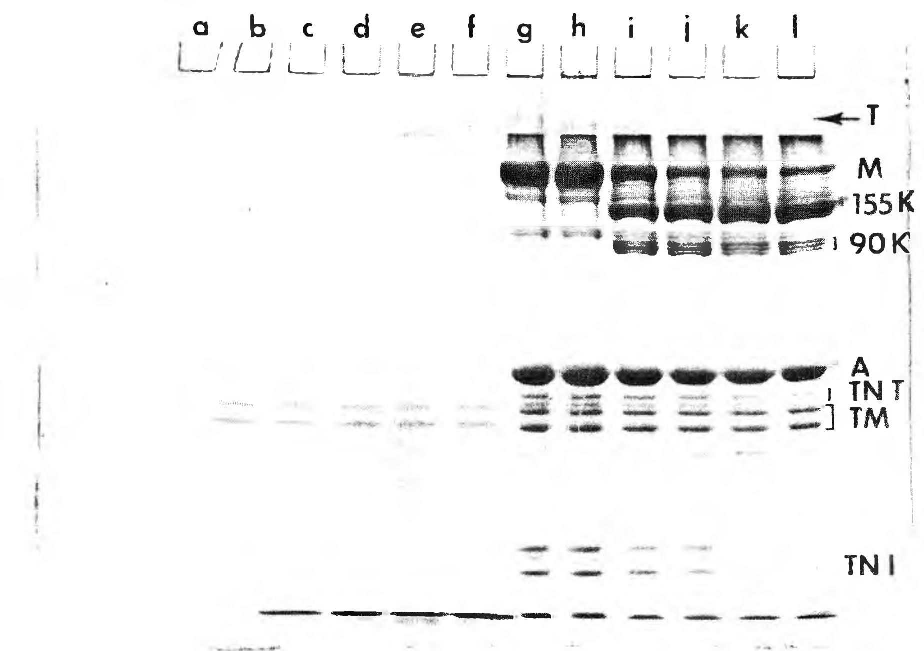

Fig. 3—Ten percent SDS-PAGE slab gel, of myofibrils incubated with purified cathepsin D at 37° C and pH 5.5. Assay conditions: Bovine skeletal myofibrils were incubated with purified cathepsin D at pH 5.5, 37° C. Lanes a through f contained 50 μL supernatant fractions corresponding to myofibrils plus pepstatin-no enzyme-control, myofibrils plus enzyme control, and 30, 60, 90, and 120 min incubations with enzyme, respectively. Lanes g through h contained 48 μg sediment fractions corresponding to myofibrils plus pepstatin-no enzyme control, myofibrils plus pepstatin plus enzyme control, and 30, 60, 90, and 120 min incubations with enzyme, respectively. Labels are: T, titin; M, myosin heavy chains; 155 K, 155,000-dalton fragment of myosin heavy chains; 90 K, a bracket indicates the position of a trio of bands of approximately 90,000 daltons; A, actin; TN T, a small bracket indicates troponin T isomers; TM, a bracket indicates the beta and alpha tropomyosin subunits; TN I, troponin I.

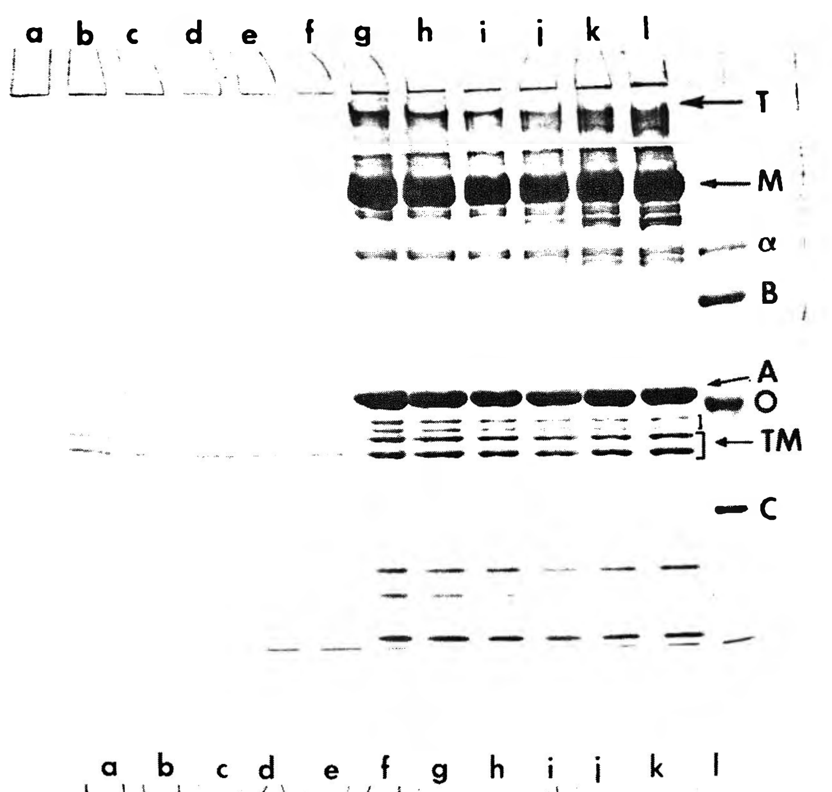

Fig. 4—Ten percent SDS-PAGE slab gel, of myofibrils incubated with purified cathepsin D at 25° C and pH 5.5. Bovine skeletal myofibrils were incubated with purified cathepsin D at pH 5.5, 25° C as indicated in Materials & Methods. Sample lanes a-f and g-l were loaded as described in Fig. 3. Labels at the right of the gel are as follows: T, position of "intact" titin; M, myosin heavy chains; α, alpha-actinin; B, bovine serum albumin; A, actin; O, ovalbumin; the small bracket indicates troponin T isomers; TM, the larger bracket indicates beta and alpha tropomyosin subunits; C, chymotrypsinogen.

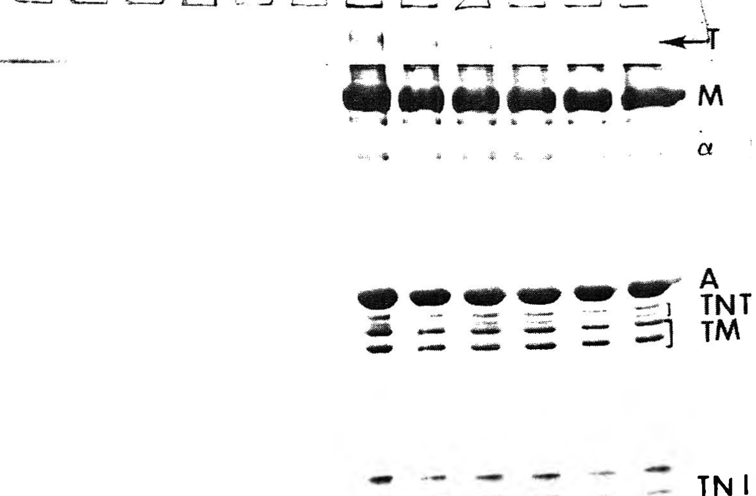

Fig. 5—Ten percent SDS-PAGE slab gel, of myofibrils incubated with purified cathepsin D at 15° C and pH 5.5. Bovine skeletal myofibrils were incubated with purified cathepsin D at pH 5.5, 15° C as indicated in Materials & Methods. Sample lanes a-f and g-l were loaded as described in Fig. 3. Labels at the right of the gel are as follows: T, titin; M, myosin heavy chains; α, alpha-actinin; A, actin; TN T, the small bracket indicates troponin T isomers; TM, the longer bracket indicates the beta and alpha tropomyosin subunits; TN I, troponin I.

from the myofibrils into the supernatant by cathepsin D (Fig. 4, Lanes a-f), and that which was released, had a very low molecular weight and migrated to the bottom of the gel (Fig. 4, Lanes d-f). The lanes (g-l) of the sediments show that some degradation occurred in the titin (compare lanes k and l with g and h). Some of the myosin heavy chains were degraded to fragments of about 155,000 and 90,000 daltons after 90 to 120 min of incubation (lanes k and l), but not nearly to the degree that had occurred at the same pH at 37° C (c.f., Fig. 3). Troponins T and I and tropomyosin subunits were only slightly degraded after 120 min of incubation.

Figure 5 contains the electrophoretic results obtained on supernatants and sediments from myofibrils incubated with cathepsin D at 15° C and pH 5.5. The supernatant lanes (a-f) show that very little protein had been solubilized in agreement with the results shown in Fig. 2. The sediment lanes (g-l) show that a significant decrease occurred in intact titin with increasing incubation time (compare lane l with lanes g and h), but no obvious degradation of myosin heavy chains or other proteins had occurred in the sediments.

Cathepsin D also was examined with respect to its effectiveness in altering the structural integrity of the myofibril under different conditions of pH and temperature. The A band regions in Fig. 6b were substantially narrowed as a result of incubation with cathepsin D at pH 5.5 and 37° C in comparison with its control (Fig. 6a). However, there was still some Z-line material left after 120 min of incubation, and there was no obvious increase in number of short (fragmented) myofibrils. After 120 min at 25°C, the A bands of the myofibrils (Fig. 6d) appeared somewhat narrow. The Z-lines may have been degraded slightly as well, but some Z-line substance remained, and there was no obvious increase in degree of fragmentation. After 120 min at 15°C, little observable change in appearance of the myofibrils had occurred. The range of con-

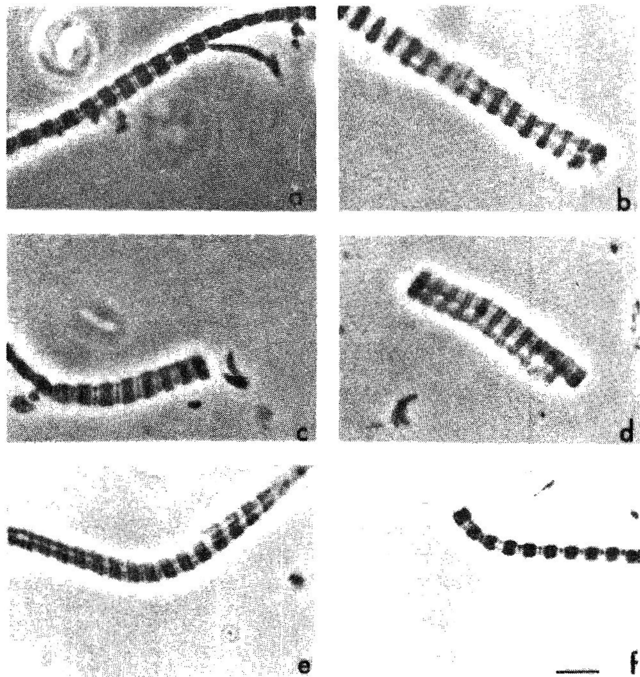

Fig. 6—Phase contrast micrographs of myofibrils after incubation with purified cathepsin D at pH 5.5 and 37, 25, or 15° C and a brief homogenization. Bovine skeletal myofibrils were incubated with purified cathepsin D as indicated in Materials and Methods. Micrographs a, c, and e represent control myofibrils containing pepstatin and no enzyme incubated 120 min at pH 5.5 and 37, 35, or 15° C, respectively. Micrographs b, d, and f represent myofibrils incubated with cathepsin D for 120 min at pH 5.5 and 37, 20, or 15° C, respectively. The bar in micrograph f represents 5.0 μm. Magnification was X2000 in all figures.

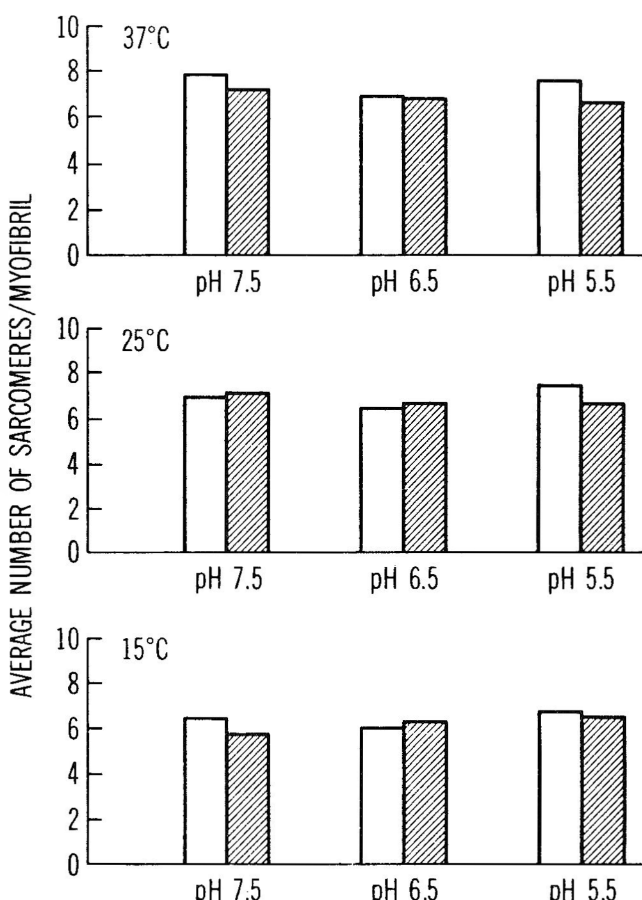

Fig. 7—Degree of myofibril fragmentation after incubation with cathepsin D at selected pH values and temperatures and a brief homogenization. Myofibrils were incubated with purified cathepsin D at 37°, 25°, or 15° C, and pH 5.5, 6.5, or 7.5 for 120 min and then subjected to a brief 10-sec homogenization. The average number of sarcomeres per myofibril was calculated and is shown as open vertical bars for the 0-min samples (myofibrils plus pepstatin-no enzyme) and as shaded vertical bars for the 120-min treated samples.

ditions in which cathepsin D was effective in causing significant myofibrillar alterations was rather narrow. Little or no effect was observed at pH 6.5 and above regardless of temperature (results not shown).

The extent of myofibril fragmentation caused by treatment with cathepsin D was difficult to assess accurately because even untreated, freshly prepared myofibrils varied widely in the number of sarcomeres per myofibril. The degree of fragmentation was measured to determine more objectively if cathepsin D treatment caused a significant increase in fragmentation. The results (Fig. 7) show the average number of sarcomeres per myofibril were not different for the control and for the 120-min samples incubated at 37°, 25°, or 15° C and at pH 7.5, 6.5, or 5.5 although in some cases extensive degradation of myofibrillar proteins had occurred. The degradation of titin shown by SDS-PAGE analysis and the lack of increase in the degree of fragmentation suggests that it and other proteins hydrolyzed by cathepsin D are not key proteins in conferring resistance to fragmentation of the myofibril.

CONCLUSIONS

THE RESULTS of this study showed that cathepsin D was active in only a limited range of pH and temperature conditions, and the alterations caused seemingly had no effect on myofibril fragmentation. In the normal course of pH decline that occurs in postmortem muscle, a pH of 5.5 would seldom be reached when the temperature is still near 37° C. If decline in pH is accelerated while the temperature remains high (e.g., by electrical stimulation) some degradation of myosin, titin, etc. could occur. However, these changes may still not cause an increase in fragmentation of myofibrils as shown here *in vitro*. These results are of importance to meat science because some investigations have proposed that cathepsin D is a causative agent in the postmortem tenderization process (Robbins et al., 1979, Dutson, 1983). In view of the *in-vitro* results presented here it seems doubtful that cathepsin D could play a principle role in this process.

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Effects of Postmortem pH and Temperature on Bovine Muscle Structure and Meat Tenderness

L. P. YU and Y. B. LEE

ABSTRACT

Bovine longissimus muscles with postmortem pH in the range 5.5 – 7.0 were subjected to different postmortem temperatures of 1°, 4°, 25° and 37°C. Intact beef sides with different postmortem pH were also subjected to two different environmental temperatures of 1° and 25°C. High pH muscles exhibited an extensive degradation of Z-lines, whereas low pH muscles showed a preferential degradation of M-lines and myosin heavy chains. Intermediate pH muscles did not show much degradation of muscle proteins, resulting in tougher meat than either low or high pH muscles. High postmortem temperatures enhanced the degradation of muscle proteins in excised and incubated muscle strips, but the delayed chilling of intact beef sides at 25°C for 8-hr did not affect either the structural changes or meat tenderness.

INTRODUCTION

MEAT TENDERNESS has been shown to be related to the ultimate muscle pH (Bouton et al., 1957, 1971, 1973), postmortem temperature (Parrish et al., 1973; Moeller et al., 1976; Pierson and Fox, 1976), the state of muscle contraction (Locker, 1960; Marsh and Leet, 1966; Smith et al., 1971) and the enzymatic proteolysis of myofibrillar proteins (Penny, 1974; Penny et al., 1974; Parrish et al., 1981; King and Harris, 1982; Suzuki et al., 1982). The mechanisms of meat tenderization during aging are still poorly understood, but it is generally agreed that postmortem pH and temperature have a great effect on muscle properties and final meat tenderness. Cold or heat shortening of muscle has been reported to have an adverse effect on tenderness (Locker and Hagyard, 1963). Beef was reported to be least tender when its postmortem pH was about 6.0 (Bouton et al., 1957). Very early high postmortem pH and temperature could enhance meat tenderness (Marsh et al., 1981). High postmortem temperature could promote degradation of myofibrillar proteins, especially myosin (Bechtel and Parrish, 1983). The degradation of myofibrillar proteins has been associated with incubation pH and temperature (Yates et al., 1983).

In most previous work (Locker and Hagyard, 1963; Davey and Gilbert, 1975, 1976; Smith et al., 1971) on beef tenderness, postmortem temperature was the only variable, and very few studies have investigated the combined effects of postmortem temperature and pH. Furthermore, it is not clear whether the results obtained from the incubation studies of excised muscles or ground meat can be directly applicable to intact beef carcasses in a commercial plant.

The objective of the present study was to investigate the combined effects of different postmortem pH and temperatures on the structural changes and meat tenderness in incubated excised muscles as well as in intact beef sides.

MATERIALS & METHODS

TWELVE STEERS of approximately 500 kg were randomly chosen. Seven out of the twelve received a subcutaneous injection of epinephrine (18.8 mg/100 kg) at 24, 16, and 8 hr prior to slaughter (treatment group). The other five steers received no injection and served as controls. Two experiments were conducted with these twelve steers.

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Table 1—Carcass traits[a]

Traits	pH <5.8	pH 5.8–6.3	pH >6.3
No. of carcasses	5	4	3
Carcass weight, kg	354 ± 27	304 ± 22	320 ± 28
Backfat thickness, cm	1.42 ± 0.55	1.57 ± 0.77	1.52 ± 0.18
Quality grade[b]	11.0 ± 1.0	13.3 ± 2.1	12.5 ± 0.7
Maturity	A	A	A
Marbling score	Small-Modest	Moderate	Modest
Color	Normal	Mod. dark	Very dark

[a] Means ± standard deviations.
[b] Avg. Prime = 15, low Prime = 14, high Choice = 13, avg. Choice = 12, low Choice = 11, high Good = 10, avg. Good = 9, low Good = 8.

Table 2—Shear value of cooked steaks from different pH carcasses[a]

Conditions	Lee-Kramer shear[b], kg/20g meat: Low pH	Intermediate pH	High pH
1 day postmortem in the cooler	149 ± 10[c]	151 ± 6[c]	95 ± 5[d]
4°C incubation			
3 day	134 ± 8[c]	158 ± 8[c]	83 ± 4[d]
7 day	108 ± 5[c]	144 ± 2[d]	75 ± 1[e]
10 day	113 ± 5[c]	135 ± 7[d]	73 ± 4[e]
25°C incubation			
3 day	111 ± 3[c]	133 ± 10[d]	78 ± 4[e]
7 day	56 ± 9[c]	84 ± 3[d]	53 ± 2[c]
37°C incubation			
3 day	107 ± 6[c]	130 ± 2[d]	80 ± 2[e]

[a] These values were obtained from excised and incubated muscle strips.
[b] Means of triplicate determination ± standard deviations.
[c,d,e] Means with different superscripts in the same row differ significantly, $P<0.05$.

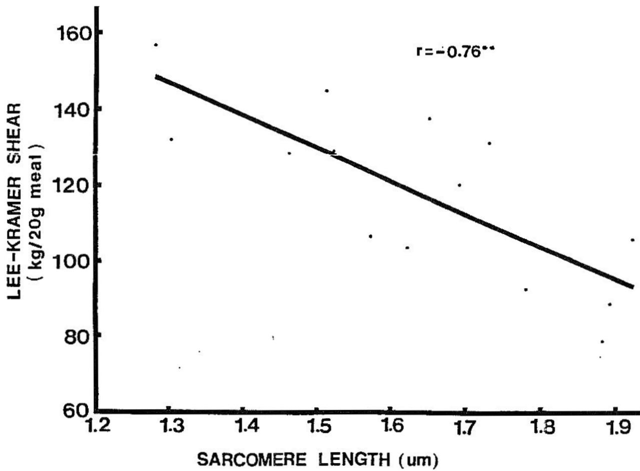

*Fig. 1—Correlation between sarcomere length and Lee-Kramer shear. Correlation coefficient was calculated from longissimus muscles of intact beef sides subjected to two different temperatures. **, $p<0.01$.*

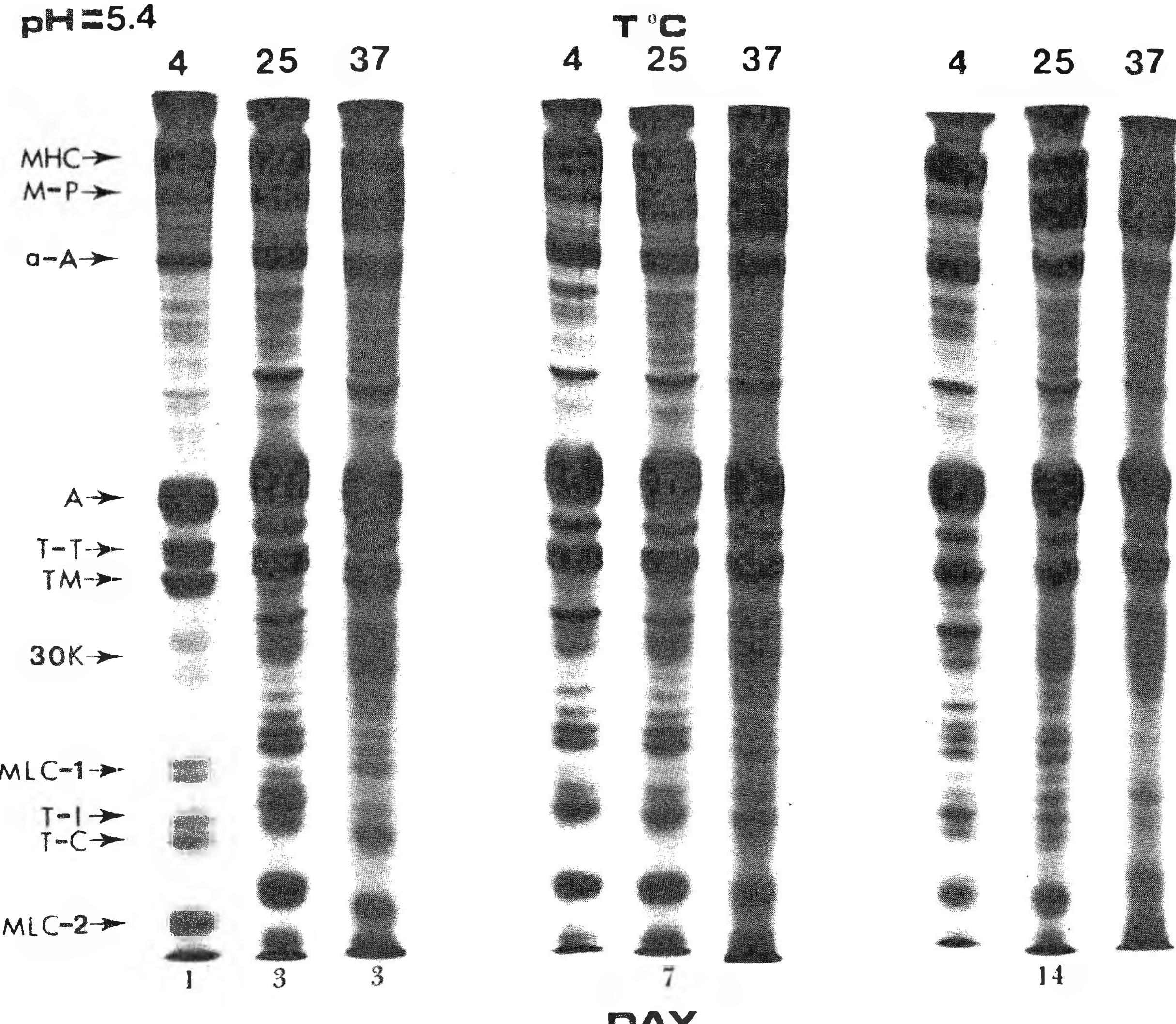

Fig. 2—Electrophoretic patterns of myofibrillar proteins from LD (longissimus dorsi) muscle of pH 5.4 incubated at different temperatures and time. MHC, myosin heavy chain; M-P, M-protein; α-A, α-actinin; A, actin; T-T, troponin T; TM, tropomyosin; 30 K, 30,000 daltons protein; MLC-1, myosin light chain 1; T-I, troponin I; T-C, troponin C; MLC-2, myosin light chain 2.

In the first experiment, four carcasses (two controls and two treated) were immediately chilled in a cooler (1°C) after slaughter. After 24 hr of chilling, carcass traits were measured and quality grades were determined by a USDA meat grader. Longissimus muscle samples were taken from each carcass at the 12th rib at 0, 4, 6, and 24 hr for pH measurement. In addition, 24 hr postmortem samples were also used for SDS gel electrophoresis. After 1 day in the cooler, a section of longissimus muscle was cut from each carcass at the 12th rib and divided into three parts. These were incubated in antibiotic saline solution (150 units of penicillin/mL, 150 units of streptomycin/mL, 60 mg Gentocin/L, and 0.9% NaCl) at 4°, 25°, and 37° C, respectively. After 1, 3, 7, 10, and 14 day incubation, samples were taken for SDS gel electrophoresis, light and electron microscopy and pH. Only those samples without bacterial growth were used. The bacterial growth was periodically monitored by culturing a portion of incubation medium in Standard Method Agar (Jensen et al., 1978).

In the second experiment, one side of each carcass (three controls and five treated) was conventionally chilled in a cooler of 1°C after slaughter (cold side). The other side was held at room temperature (25°C) for 8 hr, and then transferred to the same cooler (hot side). After 24 hr of chilling, all beef sides were moved into a holding cooler of 4°C, and carcass traits and USDA quality grade were determined. Longissimus muscle samples were taken from the 12th and 13th ribs at 1, 8 and 14 days postmortem. Sarcomere length was measured for the 1 and 8 day samples. Muscle pH was determined after 24 hr postmortem. Meat tenderness was determined for the 8 day samples. SDS gel electrophoresis and transmission electron microscopy (TEM) were used to evaluate the degradation of muscle proteins and structure for the 1, 8, and 14 day samples. Meanwhile, longissimus muscle samples taken from the cold sides at 1 day postmortem were incubated at 37°C in the same antibiotic-saline solution as described previously. After 7 days of incubation, only the samples without bacterial growth were taken from the central core of these muscles for SDS gel electrophoresis and TEM.

Measurement of pH

One gram longissimus muscle sample was homogenized in 10 mL distilled water for 1 min using a Polytron homogenizer (Brinkmann Instruments Co., Westbury, NY), then the pH value was read with a Beckman Model 3560 Digital pH Meter.

Tenderness evaluation

Two and a half cm thick steaks were cut from the primal ribs starting from the 12th rib and broiled in an oven until the internal

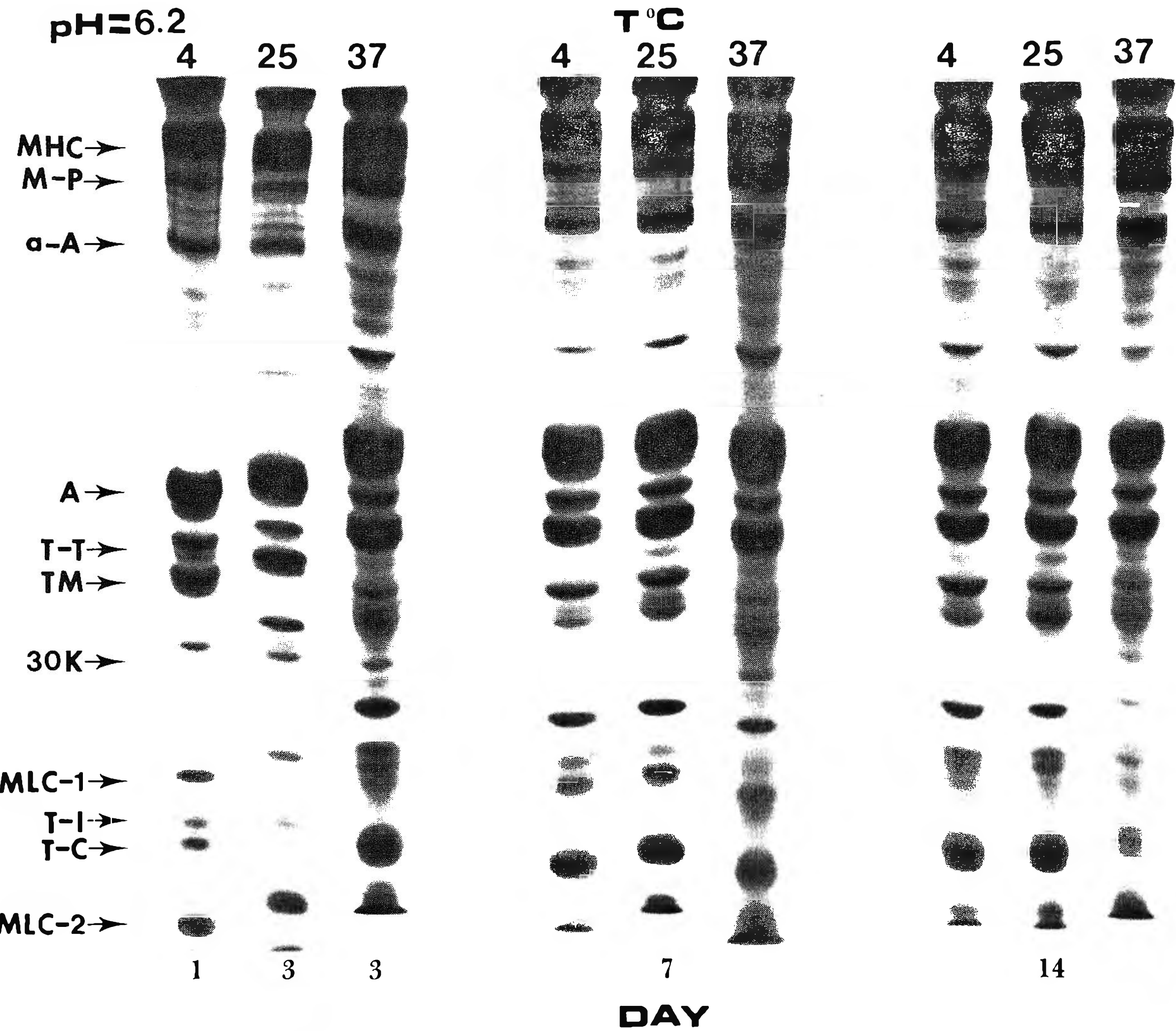

Fig. 3—Electrophoretic patterns of myofibrillar proteins from LD muscle of pH 6.2 incubated at different temperatures and time.

Table 3—Shear value and sarcomere length for cold and hot side muscles from different pH carcasses[a]

Variables	Cold side			Hot side		
	Low pH below 5.8	Intermediate pH 5.8 – 6.3	High pH above 6.3	Low pH below 5.8	Intermediate pH 5.8 – 6.3	High pH above 6.3
Shear value of cooked steaks[b]						
Warner-Bratzler, kg/core	7.4 ± 0.9[cd]	8.2 ± 1.1[d]	6.3 ± 1.1[c]	6.8 ± 0.8[c]	8.8 ± 1.0[d]	5.9 ± 1.0[c]
Lee-Kramer, kg/20g	104 ± 14[c]	142 ± 17[d]	101 ± 17[c]	109 ± 9[c]	145 ± 11[d]	104 ± 11[c]
Sarcomere length, μm	1.67 ± 0.15[c]	1.58 ± 0.18[cd]	1.60 ± 0.18[cd]	1.72 ± 0.13[c]	1.51 ± 0.16[d]	1.63 ± 0.16[cd]

[a] Means ± standard errors determined by pooled variance.
[b] Determined after 8 days of aging.
[c] Means with different superscripts in the same row differ significantly, P <0.05.

temperature reached 70°C. After cooling the cooked steaks to 25°C, three core samples of 2 cm diameter were drilled parallel to the muscle fiber from each steak for Warner-Bratzler (WB) shear measurement. In addition, the central portion of each steak was cut and coarsely ground. Twenty grams of ground meat were used for Lee-Kramer (LK) shear measurement as described by Lee (1983).

Sarcomere length measurement

Muscle samples of about 3×3×10 mm size were fixed in 10% buffered formalin solution (0.1 M phosphate buffer, pH 7.4) and blended in distilled water for about 30 sec. Then, a few drops of the homogenate were placed on the glass slides and examined with oil immersion under a Carl Zeiss light phase-contrast microscope (LPCM) as described by Varcoe and Jones (1983). In addition, the sarcomere length was also measured from the stained thick sections of TEM samples with the LPCM. The average sarcomere length was determined by measuring about 200 sarcomeres per sample.

SDS gel electrophoresis

A half gram muscle sample was homogenized for 30 sec at full speed with a Polytron in 4.5 mL of 0.05M Tris buffer (pH 7.0) and then centrifuged for 10 min. The supernatant was discarded, and the pellet was rehomogenized for 5 sec at high speed in the same Tris buffer (4.5 ml). To the homogenate, 10 mL of SDS sample buffer

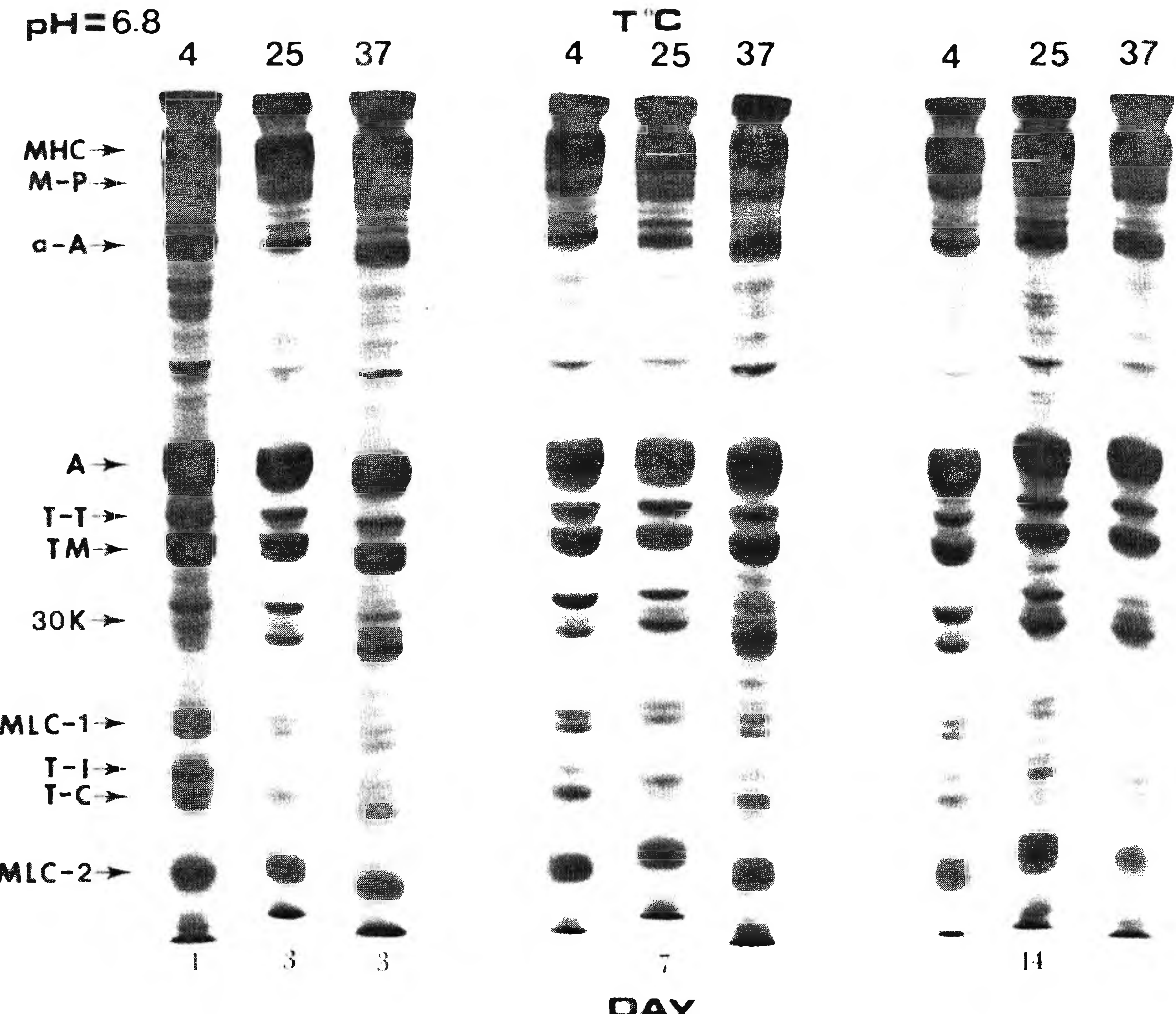

Fig. 4—Electrophoretic patterns of myofibrillar proteins from LD muscle of pH 6.8 incubated at different tempertures and time.

(4% SDS, 1.5% mercaptoethanol, 0.06M Tris, pH 6.8) was added. The whole mixture was held with agitation in a boiling water bath for 12 min. After complete solubilization of the pellets and cooling, the solution was diluted with sample buffer to contain a final protein concentration of 4 mg per mL. A stacking gel electrophoresis method described by Laemmli (1970) and modified by Lee et al. (1975) was employed. The final concentration of acrylamide was 3 and 10% for the upper and lower gel, respectively. A total of 70 μg protein was applied to each gel column and the electrophoresis was run in the presence of 0.1% SDS. The protein band was identified by the molecular weight determined against the protein molecular weight markers (Sigma Chemical Co., St. Louis, MO) according to the method of Laemmli (1970).

Transmission electron microscopy (TEM)

Muscle tissues were cut into 1 × 1 × 3 mm pieces, placed immediately in cacodylate buffered glutaraldehyde solution (1.6% glutaraldehyde, 0.16M sodium cacodylate, 0.04g $CaCl_2$/100 mL, pH 7.4) and fixed at 4°C for 2 hr. The fixed tissues were washed three times (10 min each) with cacodylate buffer (0.08M sodium cacodylate, 5.8% sucrose, pH 7.4) at 4°C. After washing, the tissue was post-fixed in buffered osmium tetroxide solution (1% OsO_4, 0.13M sodium cacodylate, pH 7.4) for 1 hr at 4°C with some agitation, then washed twice (10 min each) in the same cacodylate washing buffer. Dehydration as carried out with a series of 50, 70, 90, 95, and 100% ethanol rinses (10 min for each concentration) at 4°C. After dehydration, propylene oxide was used to transfer the sample from ethanol to the embedding medium. Finally, the muscle tissue was embedded in epoxy resin (medcast resin 152g, nadic methyl anhydride 80g, dodecenyl succinic anhydride 78g, tri(dimethylaminoethyl) phenol 5g) and heated in an oven at 60°C for 2 days. Reagents were obtained from Ted Pella, Inc., Tustin, CA.

Thick sections of 1 μm were cut from these sample blocks, stained with methylene blue azure II and examined using a Carl Zeiss LPCM. Thin sections of 50 nm were cut with a Porter-Blum MT-2 ultramicrotome, stained with 5% aqueous uranyl acetate and 0.3% alkaline lead citrate and examined with a Zeiss EM 109 transmission electron microscope operated at an accelerating voltage of 80 kv.

Statistical analyses

One-way analysis of variance among the three groups of different pH muscles for the cold and hot sides, and pair difference (cold side - hot side) analysis of variance among the three groups of different pH muscles were performed. Simple regression between the Lee-Kramer shear and sarcomere length was also calculated.

RESULTS & DISCUSSION

THE CARCASSES were divided into three different pH groups in each experiment according to their ultimate pH values: low pH (below 5.8), intermediate pH (5.8 – 6.3) and high pH (above 6.3). The three groups had similar maturity, but the intermediate pH group had a higher USDA quality grade and marbling score than the low and high pH groups (Table 1).

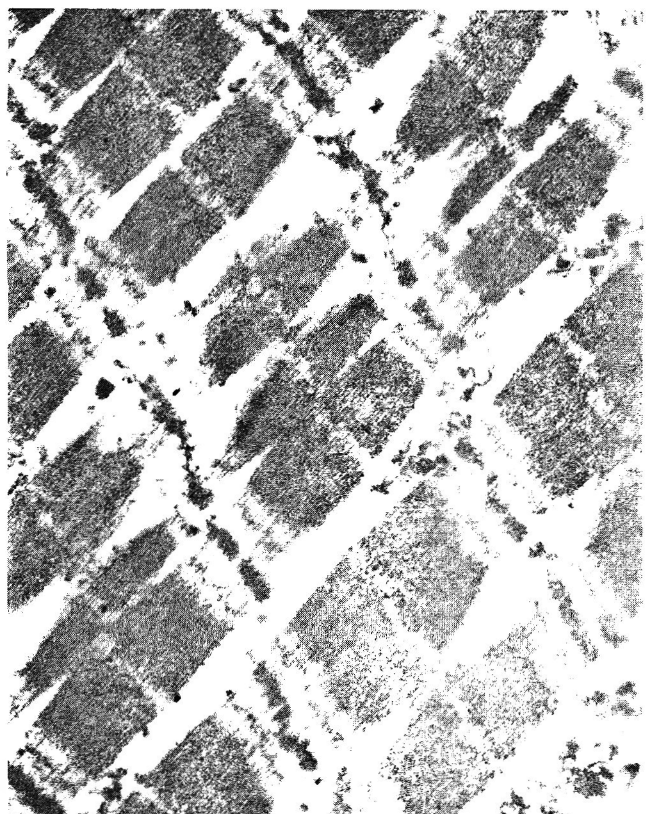

Fig. 5—A representative electron micrograph of low pH (5.4) longissimus muscle. The muscle was incubated in antibiotic saline solution at 37°C for 3 days after 1 day postmortem in a cooler, 20,300×.

Fig. 6—A representative electron micrograph of high pH (6.7) longissimus muscle. The muscle was incubated in antibiotic saline solution at 37°C for 3 days after 1 day postmortem in a cooler, 20,300x.

The high pH group produced very dark beef, whereas the low pH had normal meat color.

The effects of postmortem temperature and pH on shear values of rib eye steaks excised 1 day postmortem and incubated for different lengths of time are summarized in Table 2. When the shear was determined after 24 hr chilling in a 4°C cooler, high pH meat was significantly ($P<0.05$) more tender than low or intermediate pH meat. There was no difference between the latter two groups. At 4°C, shear values gradually decreased with incubation time for all the meats but the rate of decrease was most pronounced in low pH meat. This resulted in significant ($P<0.05$) difference in shear values after 7 days of aging among three pH groups; high pH meat was most tender, followed by low and intermediate pH meats. Elevated temperatures of 25° and 37°C further accelerated the tenderization process, resulting in identical shear values in shorter incubation periods.

The results strongly suggested that a rapid tenderization process took place during the early postmortem period (within 24 hr after slaughter) in high pH meat, whereas the tenderization process was more gradual in low pH meat. Intermediate pH meat showed the least degree of tenderization during aging, resulting in consistently tough meat regardless of aging period and temperature.

Table 3 summarizes the shear value and sarcomere length data obtained from rib eye muscles aged for 8 days in intact carcasses. As in the case of excised and incubated muscle strips, the intermediate pH muscle again showed a higher shear value than either low or high pH muscle and the average sarcomere length was shorter. Since a significant correlation between Lee-Kramer shear and sarcomere length was observed (Fig. 1), it would be expected that the intermediate pH meat would be tougher than the low or high pH meat.

These results were consistent with the early findings that meats were least tender when their ultimate pH was about 6.0 (Bouton et al., 1972, 1973). In this study, the intermediate pH carcasses had a higher USDA quality grade and marbling score than the low or high pH carcasses, and yet the meat was tougher. This suggested that the ultimate pH can be important in meat tenderness.

No significant difference was observed in sarcomere length and shear value between hot and cold sides for all the pH groups, indicating that the delayed chilling to increase early postmortem carcass temperature did not significantly affect the tenderization process during the subsequent aging.

There has been some controversy on the relationship of sarcomere length to meat tenderness. Smith et al. (1971) concluded that the degree of postmortem muscle contraction was a primary factor responsible for final meat tenderness. Other studies (Dutson et al., 1977, 1980) did not arrive at the same conclusion. In the present study, the high pH meat of the cold side carcasses had almost the same sarcomere length as the intermediate pH meat, but the high pH meat was obviously more tender than the intermediate pH meat (Table 3). Therefore, factors other than sarcomere length also contributed to the tenderization of high pH meat. Since there is a high correlation between shear force and sarcomere length as shown in this study and other studies (Locker, 1960; Marsh and Leet, 1966; Gothard et al., 1966; Smith et al., 1971; Bouton et al., 1972), sarcomere length still could be used as an indicator of meat tenderness but only for muscles with the same ultimate pH.

The results of SDS gel electrophoresis demonstrated the distinct differences in the degradation of myofibrillar proteins among muscles of different pH (Fig. 2, 3, and 4). The degradation of the myosin heavy chain (MHC) was most obvious in low pH muscle, followed by intermediate and high pH muscles. On the other hand, the degradation of troponin, tropo-

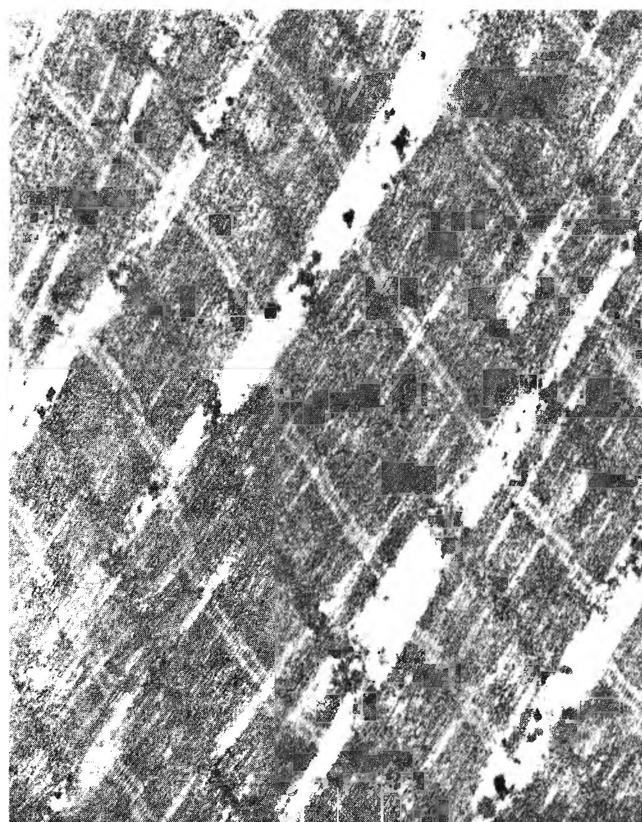

Fig. 7—A representative electron micrograph of intermediate pH (6.0) longissimus muscle. The muscle was incubated in antibiotic saline solution at 37°C for 3 days after 1 day postmortem in a cooler, 20,300x.

myosin (TM), α-actinin (α-A) which are associated with thin filament and Z-line was most pronounced in high pH muscle, followed by intermediate and low pH muscles. The results indicated that postmortem pH could dictate which myofibrillar proteins were preferentially degraded in postmortem muscle.

The electron microscopy further demonstrated a distinct difference in muscle protein degradation among three pH groups of muscles during aging. The low pH muscles (Fig. 5) shows the removal of M-lines and partial degradation of thick and thin filaments, whereas the Z-lines were preserved. Some myofibrils exhibited extensive loss of thin and thick filaments, resulting in fragmentation. In contrast, the high pH muscles (Fig. 6) showed a complete removal of Z-lines with intact M-lines. There was no convincing evidence of thick and thin filaments degradation. For the intermediate pH muscle (Fig. 7), Z-lines were well preserved, but some degradation of thick and thin filaments including an amorphous appearance of M-lines was apparent. Thus, the degradation of muscle structure observed by electron microscopy was in good agreement with the gel electrophoresis pattern.

High and low pH meats were more tender than intermediate pH meat (Tables 2 and 3). The tenderization of high pH meat was obviously due to the removal of Z-lines by neutral proteases such as calcium-dependent protease (CAF). CAF has been purified and shown to remove Z-lines and to degrade tropomyosin, α-actinin, troponin T and troponin I with concurrent production of 30,000-dalton component (Penny, 1974; Penny et al., 1974; Azanza et al., 1979; Suzuki et al., 1982). In this study, Z-lines were partially removed and the 30,000-dalton component appeared for the high pH muscle aged for 1 day in a cooler. This suggested that CAF may have been very active immediately after slaughter. The drastic removal of Z-lines (Fig. 6) and the degradation of α-actinin, troponin T, troponin I, and tropomyosin (Fig. 4) would explain the enhanced tenderness of high pH meat.

The acidic proteases like lysosomal enzymes could be responsible for the low pH meat tenderness. Some lysosomal enzymes have their optimal pH around 5.5 which is close to the pH value of the low pH muscles. They have been shown to degrade the heavy chain of myosin, α-actinin, actin, troponin T, troponin I, tropomyosin with concurrent production of the 30,000-dalton component and other small peptides (Bird et al., 1977; Schwartz and Bird, 1977; Okitani et al., 1981; Matsukura et al., 1981; Yasogawa et al., 1978). In the present study, both electrophoresis and EM showed extensive degradation of thick and thin filaments, particularly myosin heavy chain, and M-line for the low pH muscle. Therefore, the tenderization of low pH meat appeared primarily due to the proteolytic action of acidic proteases.

The intermediate pH muscles had ultimate pH in the range 5.8–6.3 which was neither the optimal pH for CAF nor for lysosomal enzymes. A very limited degradation of Z-lines and thick and thin filaments (Fig. 7) and also a limited breakdown of troponin T, troponin I, tropomyosin (Fig. 3) were shown for the intermediate pH muscles. This would explain why intermediate pH meat showed the least degree of tenderization during aging, resulting in the toughest meat.

The degradation of myosin was obvious when the muscle was incubated at high temperature for the intermediate and low pH muscles (Fig. 2 and 3). Some studies suggested that high temperature denatured myofibrillar proteins, especially myosin, were easily attacked by lysosomal enzymes to form small peptides (Schwartz ad Bird, 1977; Bird et al., 1977). However, little myosin degradation was shown for the high pH muscle even after incubation at 37°C for 8 days (Fig. 4). This suggested the importance of both postmortem pH and temperature for the postmortem degradation of muscle proteins.

Though the continuous incubation of muscle strips at the elevated temperatures accelerated the protein degradation and enhanced the meat tenderness, a brief elevation of 5° to 10°C for 8 hr after slaughter through delayed chilling of intact carcasses did not affect either the muscle protein degradation or the final meat tenderness. In addition, a high early postmortem temperature tended to cause muscle fiber shortening in some carcasses which in turn increased toughness. Lee and Ashmore (1985) also demonstrated a significant shortening of muscle fibers for the carcasses subjected to high temperature (35°C) after slaughter. Therefore, there appeared to be no beneficial effect of delayed chilling of a few hours after slaughter for the well finished carcasses unless the delay of chilling is extended to more than 1 or 2 days after slaughter.

CONCLUSION

THE POSTMORTEM pH and temperature had a significant effect on the structural changes, the degradation pattern of muscle protein and the final meat tenderness. High pH meat was most tender, followed by low and intermediate pH meats. The gel electrophoresis pattern and EM study indicated that the tenderization of high pH meat was due to the removal of Z-lines by neutral proteases, whereas the tenderization of low pH meat was due to the degradation of myosin heavy chains and M-lines by acidic proteases. Intermediate pH meat showed very limited degradation of M-lines and myosin with well preserved Z-lines. The effect of elevated temperatures above 4°C on muscle protein degradation and meat tenderness was manifested only when the incubation time extended beyond 24 hr. Holding the well finished carcasses for a few hours after slaughter at room temperature did not affect either the meat tenderness or muscle protein degradation in normal carcasses.

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Effects of Pre-rigor Pressurization, Method of Restructuring, and Salt Level on Characteristics of Restructured Beef Steaks

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ABSTRACT

Pre-rigor pressurized beef muscle was processed either by chunking and forming or flaking and forming and then mixed with either 0.00, 0.25 or 0.50% NaCl prior to the manufacture of restructured steaks. To compare the effects of pre-rigor pressurization, a post-rigor, nonpressurized, flaked and formed formulation (containing 0.50% NaCl) was also processed. Data from texture profile panel, Instron and cooking studies were collected. Steaks from pressurized muscle were similar in most characteristics to steaks processed from nonpressurized muscle. Chunking and forming produced a more fibrous, harder and cohesive steak with greater detectable connective tissue than flaking and forming. If pre-rigor pressurized beef is employed in the manufacture of restructured steaks, acceptable texture properties appear obtainable without the use of NaCl.

INTRODUCTION

PRE-RIGOR PROCESSING of beef offers potential economic advantages of reduced energy (refrigeration) input, decreased space requirements for cooling and accelerated product turnover, among others. However, utilization of pre-rigor muscle in products such as restructured meats poses a problem of cold shortening during processing. Thaw rigor may also occur during cooking if the pre-rigor boned muscle is immediately frozen and subsequent processing is done in the frozen or tempered state. Huffman et al. (1984) found restructured steaks made from hot-boned beef and processed by chunking and forming to have reduced flavor, texture and acceptability compared to restructured steaks made from cold-boned beef. Seideman et al. (1982) observed lower tenderness scores with reduced texture and flavor desirability for restructured steaks made from hot-boned, frozen and sliced beef. However, Coon et al. (1983) found that if all the restructuring processes, except pressing and slicing, occurred before freezing, then pre-rigor beef produced greater tenderness in steaks than post-rigor beef. It is conceivable that a process such as muscle pressurization may be capable of sufficient tenderizing to produce acceptable texture in restructured meats made from pre-rigor muscle. Substantial improvements in tenderness of intact muscle cuts from pre-rigor pressurized beef have been reported (Macfarlane, 1973; Macfarlane et al., 1976; Kennick et al., 1980; Riffero and Holmes, 1983).

Considerable interest is evident among consumers and processors alike for reducing sodium usage in meat processing. The total elimination of salt in restructured beef steaks has been shown to result in reduced flavor, texture and overall acceptability with increased cooking loss compared to restructured beef steaks made with NaCl (Huffman et al., 1984). Coon et al. (1983) found that increasing levels of NaCl usage (0.0, 0.5, 1.0%) resulted in higher tenderness of restructured beef steaks made from pre-rigor meat. They also noted that increased cooking yields were attained when NaCl was used with pre-rigor meat.

The objectives of this study were to determine: (1) if pre-rigor pressurized beef could produce textural and cooking properties in restructured beef steaks comparable to that achieved with post-rigor nonpressurized beef, and (2) if restructuring procedures and salt levels used with pre-rigor pressurized beef influenced textural and cooking properties of restructured beef steaks.

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MATERIALS & METHODS

TWO SEPARATE EXPERIMENTS were performed and they are outlined in Table 1. One experiment consisted of a comparison of pre-rigor pressurized muscle with post-rigor nonpressurized muscle using the flaking and forming procedure and a 0.50% salt level for both types of muscle. The other experiment compared two restructuring methods; flaking and forming and chunking and forming. For both restructuring methods, three salt levels (0.00, 0.25, 0.50%) were used. In this second experiment, only pre-rigor pressurized muscle was used.

Pressurization procedures

Two USDA Utility cow carcasses were used in this study. The inside round, outside round, eye of round, shoulder clod and inside chuck muscles from the right sides were removed within 1 hr of exsanguination. The muscles were vacuum packaged and placed in 37°C water to equilibrate muscle temperatures. Following equilibration, the muscles were placed in a preheated (35°C) chamber filled with water and subjected to 103.5 MN/m^{-2} (15,000 lb/sq in) of pressure for 2 min. The pressurized muscles and the left sides were placed in a 1°C cooler. At 72 hr post-mortem, the same muscles from the left sides were removed, vacuum packaged and all muscles were frozen at −26°C. Freezing was necessary for purposes of restructuring by flaking and forming.

Restructuring procedures

Two different steak restructuring procedures (chunked and formed, flaked and formed) were used. The round and chuck muscles were combined for each side, but kept separate for the four sides. These muscles were combined for purposes of providing a sufficient quantity of material to manufacture an adequate number of steaks. Muscles were tempered at 0°C for 24 hr after which subcutaneous fat, protruding tendons and thick connective tissue membranes were removed. Fat from U.S. Choice chucks (for fat adjustments) was either finely chopped in a bowl chopper and used later in the mixing procedure for chunked and formed steaks or flaked for use in the flaked and formed steaks. Following the restructuring procedures, the combined muscles were sampled for fat by means of a Foss-let analyzer and adjustments made to 18 ± 2% fat.

The chunking procedure consisted of grinding the meat through a 5.0 × 2.0 cm kidney-shaped plate. The combined round and chuck muscles from one of the pre-rigor pressurized sides were processed by chunking and forming. The chunked meat was divided in thirds and then mixed under vacuum in a Keebler mixer with 0.25% sodium tripolyphoshate ($Na_5P_3O_{10}$) and one of three salt (NaCl) levels (0.00, 0.25, 0.50%). The following mixing times according to NaCl level were used: 12 min for 0.00% NaCl, 10 min for 0.25% NaCl and 8 min for 0.50% NaCl. These times were selected from previous unpublished research at the U.S. Army Research & Development Center which indicated that they would provide similar bind by sight and touch.

For flaked and formed steaks, the product was processed from both pressurized and nonpressurized muscles. Muscles were tempered to −1°C and flake-cut with a Urschel Comitrol (Model 3600) using 2J030750 D flaking heat. The three NaCl levels (0.00, 0.25, 0.50%) and single $Na_5P_3O_{10}$ level (0.25%) were also added in this restructuring process for the pressurized muscle. Only the 0.50% NaCl-0.25% $Na_5P_3O_{10}$ level was used with nonpressurized post-rigor muscle. Previously mentioned mixing times according to salt level were used.

After this point in processing, common procedures were followed for both forms of restructuring. Meat was stuffed into 1.5 mil polyethylene casings using a Vemag vacuum assisted stuffer (Model Robot 100 S2). The 3.5 kg logs were frozen to −18°C and then later tempered to −1°C over an 18-hr period. The meat logs were pressed in

Table 1—Variables studied in two experiments

Experiment I[a]		Experiment II[b]						
Pre-rigor pressurization of hot-boned beef	Nonpressurization of cold-boned beef	Restructuring method =	Flaked and Formed			Chunked and Formed		
		Salt level, % =	0.00	0.25	0.50	0.00	0.25	0.50

[a] All steaks restructured by flaking and forming. All steaks processed to have 0.50% added salt.
[b] All steaks processed from pre-rigor pressurized hot-boned beef.

Table 2—Texture profile panel characteristics, procedures and definitions for restructured beef steaks

I. Visual

A. Distortion — steak is visually evaluated for the degree that the steak has warped or changes in configuration from its original raw-frozen shape. Macro distortion is degree overall steak has distorted. Micro distortion is the degree to which cooked surfaces look uneven or rough.

B. Fibrousness — steak is cut in half and the cross section is visually evaluated for the degree that the sample resembles steak or has no disruption of components.

II. Partial compression

A. Springiness — place a warm, 2.54 cm^2 piece in the mouth and, using the molars against the cooked surfaces, press lightly five times. Wait 2 sec between each press. Springiness is the perceived degree and speed with which the sample returns to original height and thickness.

III. First bite

Take a warm, 2.54 cm^2 piece and place it in the mouth in the same manner as for partial compression and evaluate for:

A. Hardness — amount of force required to bite through sample.

B. Cohesiveness — the degree to which the sample deforms before shearing.

C. Moisture release — amount of juiciness perceived during the first bite.

D. Uniformity — the degree to which the force needed to shear the sample is the same across the bite area.

IV. Mastication

Take a warm, 2.54 cm^2 sample, make the first incision as for first bite. Then turn the two pieces 90° and take a second bite. Evaluate for:

A. Sample breakdown at two chews — check the appropriate breakdown category(ies). These are identified in Table 5.

Continue chewing and evaluate for:

B. Juiciness — the amount of juice released after seven chews.

C. Size of chewed pieces — the perceived size of clearly separate pieces or pieces held together only by connective tissue web. Evaluated after 10 chews.

D. Gristle — the amount of rubbery particles present after 10 chews.

E. Cohesiveness of mass — the degree to which particles stick together. This is evaluated at its maximum degree between 10 and 35 chews.

F. Uniformity of mass — degree to which components of the mass are the same. This is evaluated after 25 chews.

G. Webbed connective tissue — amount of connective tissue present just before swallowing.

H. Number of chews — total number of chews to accurately determine the amount of webbed connective tissue.

I. Overall gristle — overall impression of the amount of rubbery particles throughout mastication.

J. Overall webbed connective tissue — amount of firm thread-like connective tissue present throughout mastication.

V. After-swallow

A. Tooth pack — amount of sample remaining in between teeth after swallowing.

B. Mouth coating — amount of film residue left on mouth surface after swallowing.

a Bettcher press (Model 70) at 500 psi using a strip loin shaped die (Bettcher shape 635). Steaks were sliced with a Bettcher cleaver (Model 39) to produce 170g steaks (1.78 cm thick). Steaks were vacuum packaged in PVDC film, frozen to −18°C and held at that temperature for 2 wk before being subjected to textural and other evaluations.

Cooking

Steaks were cooked from the frozen state on Farberware Open Hearth broilers to an internal temperature of 70°C. Steaks were turned following 12 min of cooking, at which time iron constantan thermocouples were inserted into the geometric center of each steak to monitor temperature. Steaks were given additional turning to create uniform browning. Steaks were weighed before and after cooking to determine cooking losses. Cooking times required to reach 70°C were recorded. Frozen and cooked steak width and frozen steak thickness were determined at one-quarter, one-half and three-quarters the length of the steaks. Cooked steak thickness was measured at the location of shear force measurements. Frozen and cooked steak length was determined at their maximum length values. Changes in steak thickness, width and length as a result of cooking were calculated and expressed on the basis of percentage changes. After cooking, steaks were cut and visually scored by two people for internal degree of doneness using photographic standards (8 = very rare, 1 = very well done). Sixteen steaks/formulation were subjected to cooking and configurational measurements.

Texture profile panel evaluations

A total of eight panelists comprised the texture profile panel. This panel had been involved in evaluation of restructured beef steaks for 18 months preceding this study. The various textural properties, procedures and definitions used by the panel for this product are listed in Table 2. The specific procedures used by the panel have been previously reported (Berry et al., 1985). Panelists evaluated restructured steaks from each formulation at four separate times. Approximately 1 hr was required to complete the panel determinations and discussion each time steaks from a given formulation were evaluated. On a given day, panelists met for a two-hr time period and thus evaluated steaks from two formulations.

Instron measurements

Eight frozen steaks/formulation were cooked as previously described and subjected to measurement of maximum shear force with an Instron Universal Testing Machine (Model 1122) and a straight edge shear blade (Cross et al., 1978). Each steak was sized to be 5.1 cm wide and then each section was sheared six times giving a total of 48 measurements/formulation. Steak thickness was determined before shearing at each of the six shearing sites in each steak so that maximum shear force could be expressed as Newtons/cm^2.

Statistical analysis

Data from each experiment underwent separate statistical analyses. One analyses compared the effects of pre-rigor pressurization by including the one cold-boned formulation (flaked and formed steaks made with 0.50% salt) with the corresponding pre-rigor pressurized formulation (flaked and formed steaks made with 0.50% salt). The other analyses consisted of the six pre-rigor muscle formulations involved in the two main effects of restructuring method (chunked and formed, flaked and formed) and NaCl level (0.00, 0.25, 0.50%) and the subsequent interaction of these two main effects. Analysis of variance procedures (Snedecor and Cochran, 1972) were employed. When analysis of variance revealed a significant ($P<0.05$) effect for the main effect of NaCl level, the mean separation technique of Duncan (1955) was employed.

RESULTS & DISCUSSION

TEXTURE PROFILE PANEL comparisons of flaked and formed steaks processed from pre-rigor pressurized and post-rigor nonpressurized beef are given in Table 3. Values for breakdown following two chews did not differ between the two formulations and are not given in tabular form. Flaked and formed steaks processed from pre-rigor pressurized beef had more visual micro distortion, less springiness after being partially compressed, less first bite uniformity and moisture release, lower juiciness values and greater amounts of gristle and webbed tissue than flaked and formed steaks processed from post-rigor, nonpressurized beef. The sensory panel determined that ad-

Table 3—Effects of pre-rigor pressurization of muscle on texture profile panel characteristics of resultant flaked and formed steaks

Characteristic[a]	Flaked and formed steaks from nonpressurized cold-boned beef	Flaked and formed steaks from pressurized hot-boned beef
Visual		
Macro distortion	4.4 ± 1.1	5.1 ± 1.1
Micro distortion	4.2 ± 1.0[b]	5.1 ± 1.1[c]
Fibrousness	7.1 ± 1.7	7.0 ± 1.8
Partial compression		
Springiness	9.7 ± 1.7	8.9 ± 1.8
First bite		
Hardness	8.6 ± 1.3	8.5 ± 1.7
Cohesiveness	9.6 ± 1.7	9.2 ± 1.4
Moisture release	5.8 ± 1.3[b]	4.8 ± 1.2[c]
Uniformity	11.0 ± 1.8[b]	10.1 ± 1.8[c]
Mastication		
Juiciness	7.1 ± 0.6[b]	6.1 ± 1.2[c]
Size of chewed pieces	9.9 ± 1.0	9.8 ± 1.0
Gristle	1.5 ± 1.1[b]	2.3 ± 1.2[c]
Cohesiveness of mass	9.5 ± 1.6	9.8 ± 1.3
Uniformity of mass	10.9 ± 1.4	10.8 ± 1.9
Webbed connective tissue	2.8 ± 1.4[b]	4.0 ± 1.8[c]
Number of chews	60.5 ± 9.8	59.0 ± 9.5
Overall gristle	1.9 ± 1.6	2.3 ± 1.3
Overall webbed connective tissue	2.5 ± 1.5[b]	3.6 ± 1.6[c]
After-swallow		
Toothpack	3.3 ± 1.0	2.8 ± 1.6
Mouthcoating	4.9 ± 1.3	4.2 ± 1.0

[a] Refer to Table 1 for definitions of characteristics. All values based on a 15-point scale. Characteristics expressed as means ± standard deviations.
[b,c] Means in the same row bearing different superscripts are significantly ($P<0.05$) different.

Table 4—Effects of pre-rigor pressurization of muscle on Instron values and cooking properties of resultant flaked and formed steaks

Value, property	Flaked and formed steaks from nonpressurized cold-boned beef	Flaked and formed steaks from pressurized hot-boned beef
Maximum shear force, kg	24.4 ± 2.6	24.3 ± 4.7
Maximum shear force expressed as Newtons/cm^2	28.6 ± 3.0[b]	25.6 ± 5.1[c]
Cooking loss, %	28.5 ± 2.5[b]	31.8 ± 2.2[c]
Cooking time, min/g	0.17 ± 0.02	0.18 ± 0.003
Degree of doneness score[a]	3.2 ± 0.5	3.3 ± 0.5
Change in steak thickness from raw to cooked, %	−8.2 ± 5.1[b]	+8.4 ± 6.2[c]
Change in steak length from raw to cooked, %	−22.0 ± 3.2	−25.2 ± 2.9
Change in steak width at narrow end from raw to cooked, %	+3.2 ± 6.3[b]	−8.1 ± 5.2[c]
Change in steak width in middle of steak from raw to cooked, %	+0.7 ± 6.5[b]	−4.8 ± 5.3[c]
Change in steak width at widest end from raw to cooked, %	−10.9 ± 4.2[b]	−16.7 ± 3.3[c]

[a] Degree of doneness based on an 8-point photographic scale where 1 = very well done and 8 = very rare.
[b,c] Values and properties expressed as means ± standard deviation. Means in the same row bearing different superscripts are significantly ($P<0.05$) different.

vantages previously noted for intact muscle cuts from pre-rigor pressurized beef (Macfarlane, 1973; Kennick et al., 1980; Riffero and Holmes, 1983) were partially characteristic of flaked and formed steaks. Seideman et al. (1982) found lower tenderness scores for restructured steaks processed from pre-rigor (nonpressurized) beef that was frozen prior to restructuring compared to post-rigor beef frozen prior to restructuring.

The indication of higher juiciness in steaks in this study for pre-rigor pressurized beef vs nonpressurized beef is in agreement with the findings of Macfarlane (1973). Juiciness scores in restructured steaks were not affected by hot-boning of muscle in the study of Seideman et al. (1982).

Differences in shear force attributable to pre-rigor pressurization were nonsignificant ($P>0.05$) when expressed simply as shear force (Table 4). However, when the data were expressed as Newtons/cm^2, steaks processed from pre-rigor pressurized beef had lower values than steaks made from the nonpressurized beef. This was due to steaks from the pressurized beef increasing in steak thickness during cooking, while decreases in steak thickness occurred for steaks made from nonpressurized beef. Thus, when the steak thickness values are considered (Newtons/cm^2), the shear values are less for pressurized vs nonpressurized beef. Pre-rigor pressurization has been shown to produce tremendous reductions in shear force values in meat cuts from intact muscles (Macfarlane, 1973; Kennick et al., 1980; Riffero and Holmes, 1983). An exception to this was the work of Macfarlane et al. (1981) which showed an increase in shear force values for pressurized beef muscles compared to nonpressurized control muscles. However, restraining the muscles to prevent contraction during pressurization reduced this difference considerably. Further work by Macfarlane et al. (1984) involving pressure treatment of comminuted meats led to the conclusion that the application and subsequent release of pressure may reorient certain muscle proteins resulting in increased binding. With pre-rigor boned beef that was frozen prior to restructuring, Seideman et al. (1982) found no differences in instrumental measurements of texture compared to restructured steaks made from post-rigor boned beef.

In addition to the previously mentioned increase in steak thickness during cooking, steaks processed from pressurized beef had higher cooking losses and underwent greater reduction in steak width at all measurement locations than did steaks processed from nonpressurized beef. The greater shrinkage in

Table 5—Effects of restructuring method on texture profile characteristics of restructured steaks

Characteristic[a]	Restructuring Method	
	Flaked and formed	Chunked and formed
Visual		
Macro distortion	4.8 ± 1.1[b]	5.7 ± 0.8[c]
Micro distortion	4.8 ± 1.1[b]	6.1 ± 1.4[c]
Fibrousness	5.9 ± 1.7[b]	8.3 ± 1.8[c]
Partial compression		
Springiness	8.1 ± 2.6[b]	9.2 ± 2.1[c]
First bite		
Hardness	7.9 ± 1.7[b]	9.2 ± 1.8[c]
Cohesiveness	9.0 ± 1.9[b]	10.1 ± 1.4[c]
Moisture release	4.5 ± 0.9	4.8 ± 0.7
Uniformity	10.4 ± 1.6[b]	9.8 ± 2.0[c]
Mastication		
Juiciness	5.8 ± 1.0	6.2 ± 0.9
Size of chewed pieces	9.0 ± 1.4[b]	9.8 ± 1.3[c]
Gristle	1.7 ± 1.7[b]	2.7 ± 1.8[c]
Cohesiveness of mass	9.4 ± 2.0[b]	10.6 ± 1.4[c]
Uniformity of mass	11.0 ± 1.3[b]	10.3 ± 1.0[c]
Webbed connective tissue	3.9 ± 1.2	4.2 ± 1.6
Number of chews	52.8 ± 10.8[b]	63.1 ± 6.9[c]
Overall gristle	2.0 ± 2.0[b]	2.8 ± 1.8[c]
Overall webbed connective tissue	3.6 ± 1.0	4.1 ± 1.6
After-swallow		
Toothpack	3.2 ± 0.8	2.7 ± 0.8
Mouthcoating	4.3 ± 1.2	4.1 ± 1.4

[a] Refer to Table 1 for definitions of characteristics. All values based on a 15-point scale. Characteristics expressed as means ± standard deviations.
[b,c] Means in the same row bearing different superscripts are significantly ($P<0.05$) different.

Table 6—Effects of on restructuring method on characterization of sample breakdown after two chews[a]

Description of breakdown	Restructuring method	
	Flaked and formed	Chunked and formed
Complete shearing	4.8	1.3
Incomplete shearing — threads	19.8	14.4
Incomplete shearing — crust	1.7	1.3
Complete crumbly separation	5.6	0.0
Incomplete crumbly separation — threads	19.8	1.3
Incomplete crumbly separation — crust	0.8	0.0
Compacts along shear line	18.2	6.0
Chunky and complete separation	4.8	3.5
Incomplete chunky separation — threads	16.5	49.4
Incomplete chunky separation — crust	1.7	9.6
Layered separation	3.1	1.3
Other	3.1	12.0

[a] Values are percentage frequencies of sample evaluations within product types that were classified into the breakdown descriptions.

Table 7—Effects of salt level on certain cooking properties of restructured beef steaks

Property	Salt level, %		
	0.00	0.25	0.50
Cooking loss, %	33.7 ± 2.1[a]	33.1 ± 2.1[a]	31.8 ± 2.2[b]
Change in steak width at narrow end from raw to cooked, %	+1.7 ± 5.6[a]	−4.8 ± 5.7[b]	−8.1 ± 7.3[b]

[a,b] Properties expressed as means ± standard deviations. Means in same row bearing different superscripts are significantly ($P<0.05$) different.

Table 8—Interaction effect of restructuring method and salt level on Instron values of restructured beef steaks[a]

Restructuring method	Salt level, %	Max shear force, kg	Max shear force expressed Newtons/cm²
Flaked and formed	0.00	27.5 ± 6.3	32.5 ± 8.1
Flaked and formed	0.25	24.7 ± 6.4	29.7 ± 8.1
Flaked and formed	0.50	24.3 ± 4.7	25.6 ± 5.1
Chunked and formed	0.00	27.3 ± 6.7	36.8 ± 10.9
Chunked and formed	0.25	26.3 ± 6.5	34.7 ± 8.9
Chunked and formed	0.50	34.1 ± 9.4	42.2 ± 13.3

[a] Values expressed as means ± standard deviations.

steak width of the steaks from pressurized beef is obviously partially responsible for their increase in thickness during cooking. However, a considerable amount of variation was inherent in the configurational changes in steaks from both treatments. In some studies, hot-boning has not affected cooking losses of resultant restructured products (Huffman and Cordray, 1979; Seideman et al., 1982) while in other studies, hot-boning has produced lower cooking losses than cold boning in restructured meat products (Pepper and Schmidt, 1975; Coon et al., 1983).

The effects of restructuring method on texture profile characteristics of restructured steaks made from pre-rigor pressurized beef are given in Table 5. Chunking and forming produced more macro and micro distortion in cooked steaks than did flaking and forming. As expected, chunked and formed steaks were rated as more fibrous than flaked and formed steaks. During first bite with incisors, greater hardness and cohesiveness, but less uniformity, was found for samples from chunked and formed steaks than for flaked and formed steaks. Size of chewed pieces, number of chews, gristle and overall gristle were higher in the chunked and formed steaks compared to the flaked and formed steaks. Specific comparisons in the literature for chunking and forming with flaking and forming are limited and nonexistent in the case of hot processed muscle. Booren et al. (1981) reported no differences in sensory panel scores for tenderness and connective tissue residue in steaks made by sectioning and forming vs flaking and forming, although juiciness scores were higher in steaks fabricated by sectioning and forming.

Compared to Table 5, the frequency distribution data given in Table 6 probably reflect a more descriptive situation regarding the breakdown of flaked and formed and chunked and formed steaks during minimal chewing. One of the differences between the two products, was the high frequency that panelists classified chunked and formed steaks as having incomplete chunky separation with either threads or crust, compared to flaked and formed steaks. It might be expected that chunked and formed steaks would first weaken during minimal chewing at the binding sites between the chunks. Flaked and formed steaks were categorized as having more incomplete crumbly separation with crust along with more compacting of the sam-

Table 9—Interaction effect of restructuring method with salt level on cooking properties of restructured beef steaks[a]

Restructuring method	Salt level	Cooking properties: Change in steak thickness raw to cooked, %	Cooking time, min/g	Change in steak length from raw to cooked, %
Flaked and formed	0.00	−8.4 ± 3.3	0.19 ± 0.009	−22.6 ± 3.0
Flaked and formed	0.25	−10.6 ± 5.0	0.17 ± 0.006	−19.4 ± 3.1
Flaked and formed	0.50	+8.4 ± 6.2	0.18 ± 0.003	−25.2 ± 2.9
Chunked and formed	0.00	−17.7 ± 7.4	0.18 ± 0.021	−22.2 ± 3.6
Chunked and formed	0.25	−17.2 ± 5.4	0.19 ± 0.012	−24.2 ± 5.0
Chunked and formed	0.50	−15.8 ± 4.6	0.18 ± 0.008	−21.6 ± 2.4

[a] Values expressed as means ± standard deviations.

ple along the shear line of the teeth. Description of breakdown after two chews did not appreciably differ according to NaCl levels.

Salt levels exerted no effects ($P>0.05$) on texture profile panel characteristics. Previous studies (Schwartz and Mandigo, 1976; Huffman et al., 1981, 1984) have shown that increased NaCl levels produced either improvements in panelists' ratings for tenderness or texture desirability in restructured steaks and chops manufactured from post-rigor muscle. In our study, regardless of NaCl level, $Na_5P_3O_{10}$ was used (0.25%). This could have produced some effects on textural properties, although Cardello et al. (1983) found in the absence of NaCl, that $Na_5P_3O_{10}$ produced only an increase in the perception of moisture/oil release from restructured steaks.

Steaks processed with 0.50% NaCl had slightly lower cooking losses than steaks processed with 0.00 or 0.25% NaCl (Table 7). Increasing levels of NaCl have been shown to reduce cooking losses for restructured beef steaks and pork chops (Schwartz and Mandigo, 1976; Huffman et al., 1981, 1984). In our study, as NaCl usage increased, the amount of shrinkage in width during cooking at the narrow end of the steak increased.

Shear force and Newton values were affected by a significant ($P<0.05$) interaction involving restructuring method and NaCl level (Table 8). The use of 0.50% NaCl resulted in lower shear force and Newton values for flaked and formed steaks, but higher shear force and Newton values for chunked and formed steaks compared to the other treatments. In the case of Newtons, the lower values for flaked and formed steaks containing 0.50% NaCl may be due to the increase in steak thickness following cooking, while the other formulations decreased in steak thickness as a result of cooking (Table 9). Several studies (Huffman et al., 1984; Coon et al., 1983) have considered the process of chunking and forming of hot-boned beef for steaks using various NaCl levels and completing the processing procedures before freezing. Huffman et al. (1984) found that regardless of the rigor state, the use of 0.75% NaCl vs no NaCl produced higher shear force values. Coon et al. (1983) also found that higher salt levels with both pre- and post-rigor beef produced higher Instron break force values in chunked and formed steaks. The effects of using hot-boned, then frozen beef in conjunction with various NaCl levels in flaked and formed beef steaks, has not been previously reported.

The use of 0.25% NaCl required fewer min/g cooking time and produced less reduction in steak length during cooking compared to 0.00 and 0.50% NaCl levels. The opposite was true regarding NaCl levels in chunked and formed steaks (interaction significant at $P<0.05$).

CONCLUSIONS

RESULTS from this study reflected only minimal differences in textural and cooking properties of restructured steaks made from pre-rigor pressurized beef vs those obtained from steaks made with nonpressurized post-rigor beef. However, in view of the reported reductions in tenderness and texture from using pre-rigor (nonpressurized) beef in restructured steaks, the pressurization procedure used in this study may indeed be improving tenderness and texture. With pre-rigor pressurized beef, the process of chunking and forming appeared to produce a more fibrous, harder and more cohesive steak with greater amounts of detectable connective tissue than flaking and forming. Since the use of NaCl failed to produce any detectable differences in texture as measured by a texture profile panel, it is possible that regardless of the restructuring method, NaCl may not be necessary for producing acceptable restructured steaks from pre-rigor pressurized beef.

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Effect of Heat Treatment on Preparation of Colorless Globin from Bovine Hemoglobin Using Soluble Carboxymethyl Cellulose

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ABSTRACT

Optimum conditions for the preparation of colorless globin using soluble carboxymethyl cellulose (CMC) were established by mapping super-simplex optimization. Response for minimization was calculated using two parameters of heme content and protein recovery. When three factors (initial pH, CMC concentration, and final pH) were used, the minimum response of 15.4 was obtained. However, much lower minimum response (8.6 or 6.2) was obtained by adding urea or by heating in addition to the above three factors. By determining the effects of heating temperature (20–80°C) at different final pH (2.25–3.06), the heme content of globin obtained from heated hemoglobin was remarkably lower (2.6–5.8%) than from unheated hemoglobin (29.2–70.7%).

INTRODUCTION

ANIMAL BLOOD collected in a slaughter house contains about 18% protein, of which about 60% is found in the blood corpuscles. Although hemoglobin, accounting for most of the solids in blood corpuscles, is a valuable source of edible protein, its utilization is limited at present because of the dark reddish color and objectionable flavor.

Decolorization can be achieved through the separation of hemoglobin into heme and globin fractions using acidified acetone (Jope et al., 1949; Tybor et al., 1975). Recently, Sato et al. (1981) developed a simple method for the preparation of colorless globin using carboxymethyl cellulose (CMC) column chromatography. Autio et al. (1983) showed that soluble CMC also can be used for the decolorization of hemoglobin. The functional properties, prepared by the procedures mentioned above, were characterized by Tybor et al. (1973, 1975), Crenvelge et al. (1974), Knapp et al. (1978), Hayakawa et al. (1982, 1983) and Autio et al. (1984, 1985).

The method using soluble CMC has the advantage of low cost compared to the chromatographic method, but the globin preparations contain a considerable amount of heme because of residual salt after adjustment to low pH. The CMC-heme and protein-heme interactions are competitive at low pH, and the latter is relatively stronger than the former in the presence of salt. Therefore, more efficient decolorization can be expected if the heme-protein interaction can be reduced by denaturation of hemoglobin.

The purpose of the present study was to determine the optimum conditions for the preparation of colorless globin using soluble CMC by mapping super-simplex optimization and to assess the effect of denaturation of hemoglobin on decolorization.

MATERIALS & METHODS

Materials

Bovine hemoglobin (H2500), porcine hemoglobin (H4131) and low viscosity carboxymethyl cellulose (CMC) sodium salt (C8758) were purchased from Sigma Chemical Co. (St. Louis, MO).

Decolorization of hemoglobin

Hemoglobin was dissolved in distilled water (Experiments A and C) or urea solution (Experiment B) at the concentration of 5 g/100 mL, and pH was adjusted to an appropriate value (initial pH) with 1N HCl. In Experiment C, the hemoglobin solution was heated at an appropriate temperature for 10 min. Then, an equal volume of CMC solution was mixed with the hemoglobin solution, and pH was adjusted to the final pH immediately. In Experiment B, the mixed solution was diluted with four volumes of distilled water followed by adjustment to final pH. After stirring for 30 min at 20°C, the mixture was centrifuged at about 1000 × *g* for 10 min. The volume of supernatant (decolorized hemoglobin solution) was measured, and protein content was determined by the method of Lowry et al. (1951). Absorbance at 380 nm of the supernatant was also determined.

Measurement of heme content and protein recovery

Protein recovery (PR) was calculated as follows; PR = (CsVs/CoVo) × 100, where Cs = protein concentration (g/100mL) of the supernatant, Vs = volume (mL) of the supernatant, Co = protein concentration of the hemoglobin solution, and Vo = volume of the hemoglobin solution. The theoretical PR value of 100 can be expected when 100% heme is removed.

Heme content (HC) was calculated as follows. HC = (As/Ao) × 100, where As and Ao are the absorbances at 380 nm of supernatant and hemoglobin solution, respectively, at the protein concentration of 1 mg/ml.

Calculation of response for optimization

Response (RE) for mapping super-simplex optimization was calculated from heme content (HC) and protein recovery (PR) by the following equation: RE = $(HC/PR^2) \times 10{,}000$. Excessively low protein recovery of globin was undesirable even if the heme content was considerably low, so that it was decided to divide HC by PR^2.

Mapping super-simplex optimization (MSO)

The MSO procedure developed by Nakai et al. (1984) was performed using a Sharp PC 2500 hand-held computer with 21K RAM. In Experiment A, the initial pH, CMC concentration and final pH, with the lower and upper limits of 1.5 and 3, 0.25% and 1.0%, and 2 and 3, respectively, were used as factors. In Experiment B, initial pH, CMC concentration, the final pH and urea concentration having lower and upper limits of 2 and 7, 0.25% and 1.0%, 2 and 3, and 1M and 8M, respectively, were used as factors. In Experiment C, the initial pH, CMC concentration and final pH and heating temperature with lower and upper limits of 1.5 and 7, 0.25% and 1.0%, 2 and 3, and 50°C and 80°C, respectively, were used as factors.

Statistical analysis

Multiple regression analysis was carried out with the SAS package program (SAS, 1982), while three-dimensional surface plots were drawn using the SAS/Graph procedure (SAS, 1982) with a Fujitsu Facom M-382 computer.

RESULTS & DISCUSSION

MSO for globin preparation

Colorless globin was successfully prepared by the CMC chromatographic method developed by Sato et al. (1981). Extremely low response (RE about 4.2) was obtained by this

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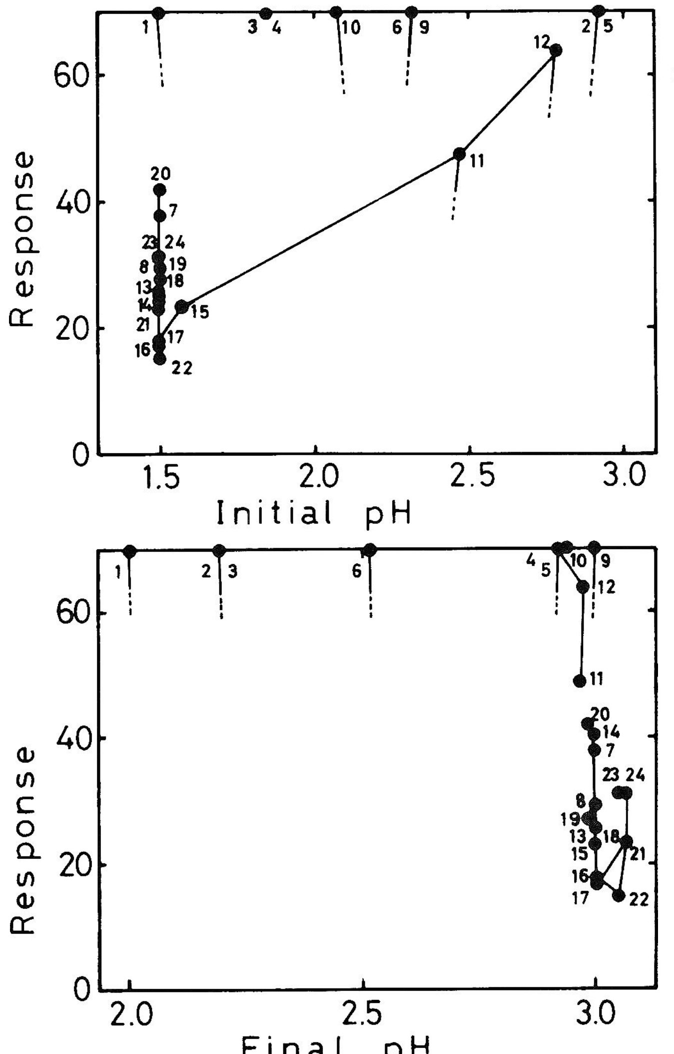

Fig. 1—Mapping results including those of simultaneous factor shift for experiment A to minimize the response for preparing colorless globin. Range initially set for optimization: initial pH 1.5–3.0, CMC concentration 0.25–1.0%, and final pH 2.0–3.0.

Table 1—Recommended conditions for the preparation of colorless globin from bovine hemoglobin using low viscosity CMC

Exptl. no.	Conditions: Initial pH	CMC conc (%)	Final pH	Urea conc (M)	Heating temp (°C)	Response[a]	Heme content (%)	Protein recovery (%)
A-22	1.50	0.96	3.06	—	—	15.4	2.6	41
B-15	5.87	0.92	2.82	6.2	—	8.6	4.4	72
C-12	2.09	0.25	2.83	—	75	7.2	4.7	81
C-17	1.50	0.72	2.25	—	77	6.2	5.6	95
C-18	1.50	0.77	3.00	—	80	12.2	1.7	37
C-23	1.50	0.77	2.68	—	75	6.8	2.5	61

[a] [Heme content/(Protein recovery)^2] × 10^4

chromatographic method, since heme content and protein recovery for the globin eluted by 0.01N HCl were 2% and 69%, respectively. However, much higher RE (about 30) was obtained when the globin was eluted with 0.01N HCl containing 0.1M NaCl. Typical globin preparations obtained by Autio et al. (1983) using soluble CMC contained 0.07% Fe representing 20% heme. RE for the method using soluble CMC was also calculated to be more than 20. Therefore, decolorization methods using CMC chromatography and soluble CMC in the presence of salt left considerable amounts of heme in the globin preparations. The optimum conditions which yielded RE values in the same order as 4.2 (target value) were expected in this study.

Several optimization designs, e.g., multiple regression approach (Schutz, 1983), response surface methodology (Box et al., 1978), and simplex methods (Morgan and Deming, 1974; Routh et al., 1977; Nakai et al., 1984) were introduced to optimize food processing and production. Nakai et al. (1984) suggested that mapping super-simplex optimization was the most efficient procedure among them. Therefore, it was reasonable to use this simplex procedure to optimize the conditions for the preparation of colorless globin.

In Experiment A, three factors (initial pH, CMC concentration and final pH) with predetermined lower and upper limits were used for decolorizing hemoglobin without heat and urea treatments. Figure 1 shows mapping of the responses including those of the simultaneous factor shift as functions of each factor. The initial pH for optimum RE may be at or below 1.5, since most experimental conditions for the initial pH have converged on pH 1.5. However, the initial pH was constrained at 1.5 to avoid excessive hydrolysis of protein with acid and a large amount of salt after neutralization. Low response values

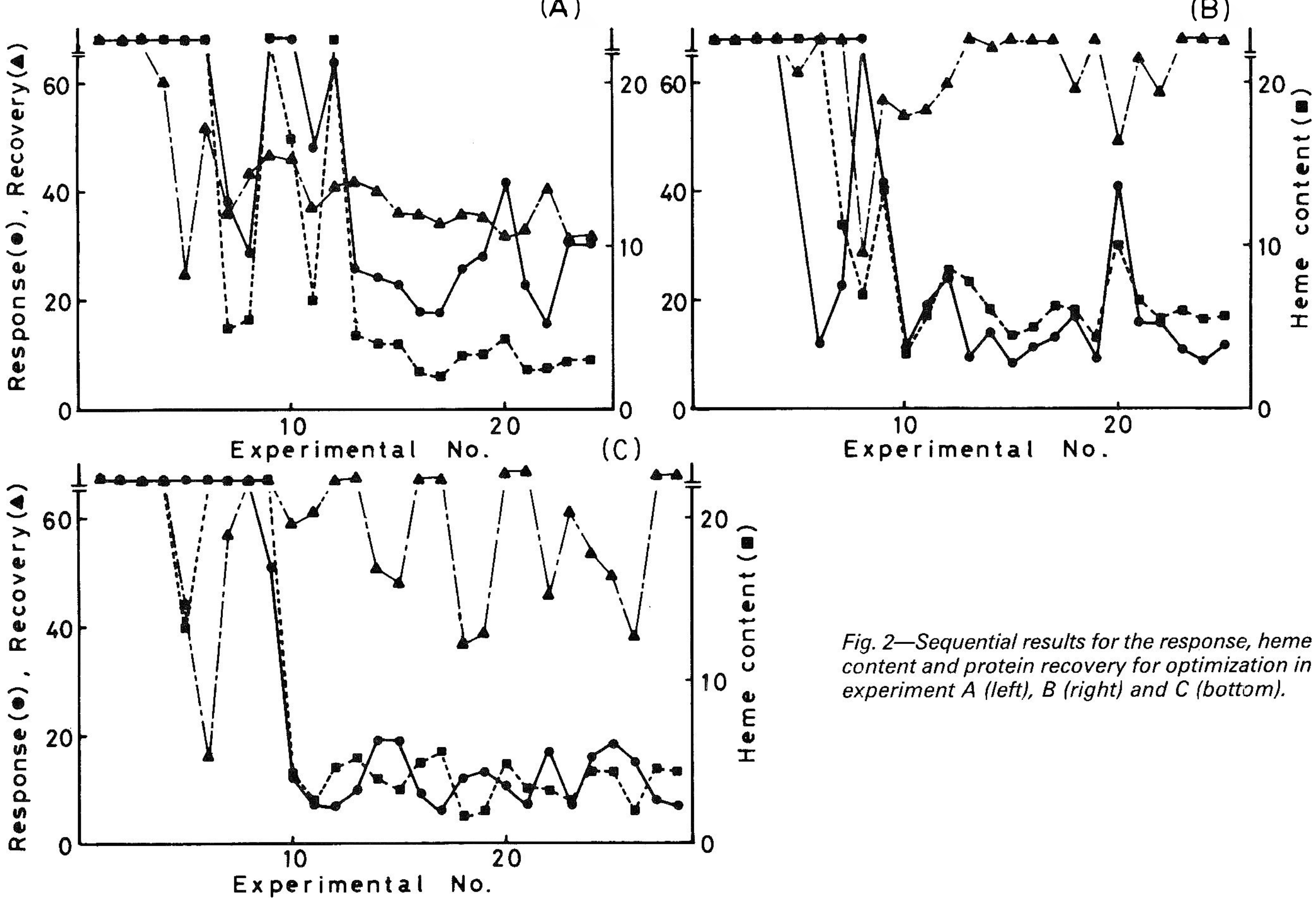

Fig. 2—Sequential results for the response, heme content and protein recovery for optimization in experiment A (left), B (right) and C (bottom).

were obtained within a narrow range of CMC concentration (0.85–1.0%) and final pH (3.0–3.1).

Figure 2A shows plots of the response, protein recovery and heme content against the experimental number. MSO, after 24 experiments, showed the minimum RE (15.4) at initial pH 1.5, CMC concentration 0.96% and final pH 3.06 (Table 1). However, RE derived from the experiment performed at optimum conditions was considerably worse than the target value (4.2) due to low protein recovery, although the globin preparations contained only a low level of heme (1.7–2.6%). The product may be still acceptable as a food ingredient since decolorization was successful.

In Experiment B, hemoglobin was treated with different concentrations of urea. Figure 3 shows the mapping of the responses including those of simultaneous factor shift against each factor. Mapping indicated that low response values could be obtained over a wide range of initial pH and CMC concentration, which meant that initial pH and CMC concentration were not important factors. On the other hand, responses showed low values at final pH above 2.8 and at urea concentration above 4M, indicating that these two factors were quite important.

Figure 2B shows the response, protein recovery and heme content plotted against experimental number. Considerably low responses of less than 10 were obtained after experiment 10. These low response values were attributed to high protein recovery. The minimum response was shown at initial pH 5.87, CMC concentration 0.92%, final pH 2.82 and urea concentration 6.2M (Table 1). The response at the optimum, 8.6, is close to the target value. Therefore, unfolding of protein prior to CMC treatment was considered to be useful in improving the response. However, the heme content of the globin preparations even at the optimum conditions was slightly high.

Experiment C was performed using heat denatured hemoglobin. Figure 4 shows the mapping of the responses for Experiment C including those of simultaneous factor shift against each factor. Low response values were obtained at initial pH below 2, especially 1.5, but initial pH was constrained at 1.5 for the same reasons as in Experiment A. Concentration of CMC was considered to be a less important factor within the range used, since low responses were obtained at CMC concentration with a wide range from 0.25–0.8%. Heat treatment was considered to be quite an important factor for preparing colorless globin, since low responses were obtained from hemoglobin heat treated at above 70°C. Final pH giving low responses varied from 2.2 to 3.0. However, final pH was still an important factor in Experiment C, because a reasonably high protein recovery was obtained at final pH below 2.5, while at final pH near 3, globin preparations contained a very low level of heme.

Figure 2C shows the response, protein recovery and heme content for Experiment C plotted against experimental numbers. MSO, after 28 experiments, showed two minimum responses close to the target value at initial pH 1.5, CMC concentration 0.72%, final pH 2.25 and heating temperature 77°C (Table 1, Experiment C-17) and initial pH 1.5. CMC concentration 0.77%, final pH 2.68 and heating temperature 75°C (Table 1, Experiment C-23). Heme content of the globin obtained under the former conditions (5.6%) was much higher than that under the latter conditions (2.5%). Therefore, the conditions for experiment C-23 were considered more effective than that for Experiment C-17. Other recommendable conditions for the preparation of colorless globin were also listed in Table 1. In Experiment C-11, only a small amount of CMC was required for the decolorization, and in experiment C-18, the globin with the lowest heme content was obtained.

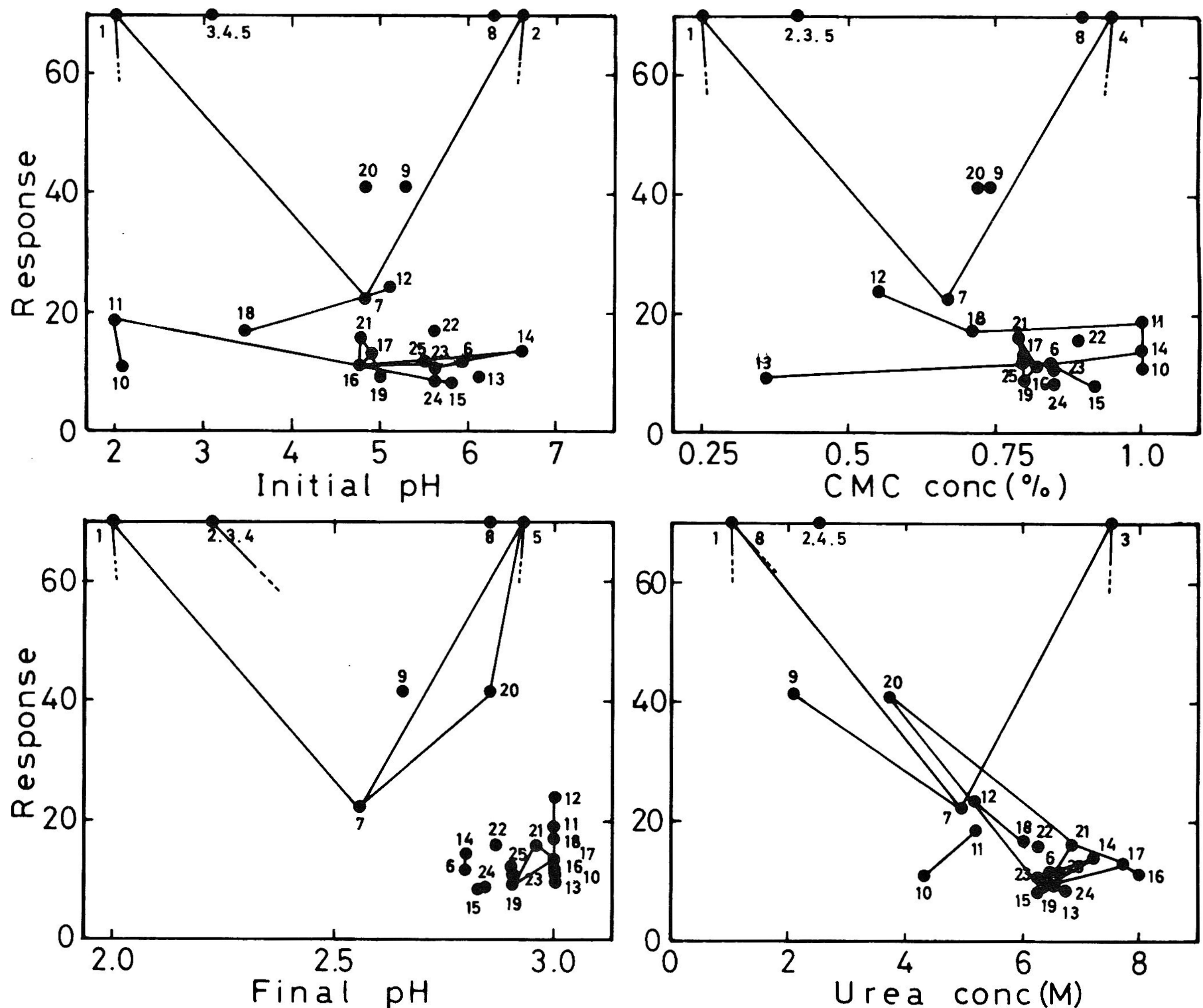

Fig. 3—Mapping results including those of simultaneous factor shift for experiment B to minimize the response for preparing colorless globin. Range initially set for optimization; initial pH 2.0–7.0, CMC concentration 0.25–1.0%, final pH 2.0–3.0, and urea concentration 1–8M.

Table 2—Effect of heat treatment on the preparation of globin using low viscosity CMC[a]

pH	Temp (°C)	Response	Heme content	Protein recovery
2.25	20	76.4 ± 0.7	70.7 ± 3.8	96.2 ± 3.0
	50	52.1 ± 1.6	27.9 ± 1.6	74.4 ± 3.6
	65	26.3 ± 4.7	13.8 ± 2.6	72.5 ± 0.1
	80	9.1 ± 1.4	5.8 ± 0.7	81.8 ± 4.3
2.68	20	95.6 ± 4.1	42.3 ± 1.6	66.5 ± 1.3
	50	43.8 ± 4.2	12.7 ± 1.6	53.7 ± 0.8
	65	10.2 ± 0.4	4.1 ± 0.4	63.2 ± 1.9
	80	9.2 ± 0.4	3.6 ± 0.2	67.4 ± 5.9
3.06	20	128.7 ± 14.9	29.2 ± 4.8	47.6 ± 4.5
	50	42.4 ± 2.6	4.0 ± 0.3	30.7 ± 0.5
	65	14.3 ± 0.4	2.6 ± 0.1	42.6 ± 0.2
	80	12.9 ± 1.2	2.6 ± 0.1	45.1 ± 1.3

[a] Mean values of three replications and standard deviation

Effect of heat treatment

Multiple regression analysis for 28 experiments in MSO experiment C showed that heating temperature significantly ($P<0.01$) influenced the response. Therefore, the effect of heating was further determined. Table 2 shows the response, heme content and protein recovery when decolorization was performed using heating temperature and final pH as factors at initial pH 1.5 and CMC concentration 0.5%. The responses were remarkably improved by heat treatment of hemoglobin above 65°C at any final pH. The remarkable decrease of the response value by heating was mainly due to the appreciable decrease of heme content. Multiple regression analysis of data of 12 experiments showed a highly significant correlation of heating temperature (TP) and final pH (pH) to heme content (HC). The following equation was derived for HC:

$$HC = 401.7 - 4.08\ TP + 0.012\ TP^2 - 174.7\ pH + 20.1\ pH^2 + 0.81\ TP\ pH$$
$$(R^2 = 0.997,\ p<0.001)$$

Figure 5 illustrates the three-dimensional surface plots to visualize the relationship between heating temperature, final pH and heme content.

The iron in the heme is linked at two sites to the histidine imidazole nitrogen atoms of the polypeptide chains, and the heme group is embedded in the interior of the hydrophobic pocket. The possibility of splitting hemoglobin at low pH into heme and the protein moiety has been known (Wymann 1948; Rossi-Fanelli et al., 1964). Therefore, the remaining heme groups in the globin prepared with unheated hemoglobin may result by retention of the heme in the hydrophobic pocket of the protein even after the linkage between the iron and imidazole groups has been split. Possibly, in the present study, the

Fig. 4—Mapping results including those of simultaneous factor shift for experiment C to minimize the response for preparing colorless globin. Range initially set for optimization: initial pH 1.5–3.0, CMC concentration 0.25–1.0%, final 2.0–3.0, and heating temperature 50–80°C.

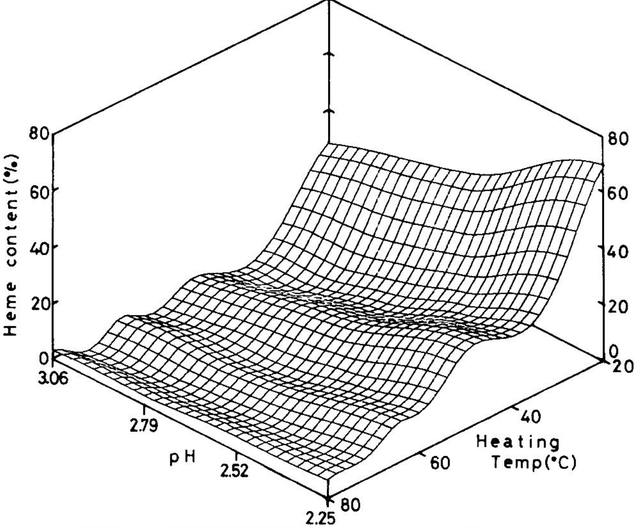

Fig. 5—Three-dimensional surface plots of the heme content as functions of heating temperature and final pH.

heme group was released by thermally induced unfolding of the protein molecule when hemoglobin was heated at pH 1.5, and then, the heme group coprecipitated with CMC.

In conclusion, MSO showed the minimum response (15.4) at initial pH 1.5, CMC concentration 0.96% and final pH 3.06, when unheated hemoglobin was decolorized. The response was considerably improved by heating or urea treatment. The optimum conditions to obtain the minimum response (6.2) for heated hemoglobin were as follows: initial pH 1.5, CMC concentration 0.72%, final pH 2.25, and heating temperature 77°C. It was suggested that thermally induced unfolding of protein around the hydrophobic pocket promoted the binding of heme group to CMC molecules.

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Some Effects on the Mechanical Properties of Meat Produced by Cooking at Temperatures between 50° and 60°C

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ABSTRACT

Peak force (PF) shear values obtained for stretched muscles from beef animals of three ages (2-3 months, 2-6 year and 12-17 years) decreased when heated for 1 hr at temperatures above 50°, 55° and 60°C respectively. PF values obtained for veal muscles were unaffected by heating at 50°C for up to 8 hr but rapidly decreased with increased time of heating at 55° or 60°C. The decrease in shear values with heating time was still evident after the connective tissue contribution was eliminated by a further cook at 80°C. Samples from the oldest animals required 24–48 hr at 60°C to produce a large decrease in the connective tissue contribution. Tenderization by prolonged cooking at 50–60°C was achieved by accelerated aging of the myofibrillar structure and, at ≥ 55°C, by a weakening of the collagenous connective tissue also.

INTRODUCTION

MUCH of the earlier literature on factors affecting tenderness during the cooking of meat has been reviewed by Laakkonen (1973). Laakkonen et al. (1970) concluded that the major decrease in Warner-Bratzler (WB) peak shear force values obtained when beef muscles were heated at a slow rate occurred at 50–60°C. This confirmed earlier work (Machlik and Draudt, 1963; Tuomy et al., 1963; Draudt et al., 1964) which showed that a large decrease in shear force values occurred between 50 and 65°C. Other workers (Bramblett et al., 1959; Bramblett and Vail, 1964; Bayne et al., 1969) reported that cooking meat by holding in an oven at relatively low oven temperatures (<120°C) for up to 30 hr produced meat more tender than that cooked at the usual higher temperatures. It was also found (Bramblett et al., 1959) that the length of time the meat was held at an internal temperature of 57–60°C was correlated with tenderness ($R^2 = 0.53$). Recent studies in Norway (Braathen, 1983) have shown that tenderness can be improved by heating meat in an oven at 70°C for 12–16 hr to give internal temperatures of 64–66°C.

Penfield and Meyer (1975) found that significantly more collagen was solubilized during a slow rate of heating to 70°C than with a fast rate but concluded that part of the difference may have involved increased proteolysis of the myofibrillar proteins. Measurement of aging rates (Davey and Gilbert, 1976) indicated that the rate increased exponentially up to 40°C, rose more slowly to a maximum at 60°C and then decreased sharply to approach zero at 75°C. Davey and Niederer (1977) have suggested that tenderization of meat by cooking at temperatures of up to 100°C takes place (a) by specific proteolytic attack on the myofibrillar structure at temperatures up to 65°C and (b) by destruction or weakening of the collagen in the connective tissue at temperatures above 70°C. There is some evidence (Bouton and Harris, 1981) that increasing the cooking time from 1 to 24 hr for meat samples heated at temperatures in the range 50–65°C has a large effect on connective tissue strength. Heating meat at 50–65°C for an extended time could thus be doubly beneficial by reducing both myofibrillar and connective tissue contributions to toughness. If the connective tissue could be weakened via a pre-cooking heat treatment, then, provided suitable precautions were taken to avoid pre-rigor toughening of the myofibrillar structure, such a treatment could be used to upgrade the quality by improving the tenderness of meat whose toughness was largely attributable to connective tissue.

In earlier work (Bouton and Harris, 1972, 1981; Bouton et al., 1974, 1981) WB shear force curves were obtained for stretched muscles heated to 40–95°C from animals of estimated ages. The stretching was to avoid toughness due to myofibrillar contraction. In this present work (a) animals of known age have been used and (b) the effect of various pre-cooking treatments on the tenderness of meat from animals of different age groups have been investigated.

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MATERIALS & METHODS

Animals and muscle treatments

In Experiment 1, the effects of animal age were assessed. Three age groups (steers aged 12–17 yr, 2–6 yr and calves aged 2–3 months) were used with eight animals in each group. The ages of all these animals were known to within a week whereas in many previous experiments, ages have been estimated from dentition. In Experiment 2, the effects of age vs heating temperature and time were investigated. Material for this experiment was obtained from the old animals in Experiment 1 plus four calves (2–3 months). All carcasses were hung within 1 hr of slaughter from either the pelvis (for the calves) or from the sacrosciatic ligament (for the beef sides). All carcasses/sides were held at 0–1°C for 48 hr before the semimembranosus (SM) muscles were removed from one side of each carcass, for the older animal, or from both sides, for the calves. After dissection, each SM muscle was trimmed of extraneous fat and obvious external connective tissue and the two end pieces removed leaving the mid sections (representing 60–70% of the muscle length) which were used in Experiment 1. These sections, together with the end pieces from the 17 yr old animals (used for Experiment 2), were then frozen and stored at −32°C until required. When required, they were thawed by holding overnight at 6–7°C.

In Experiment 1 each pair of veal SM muscles and each SM muscle sample from the other age groups were thawed and then subdivided to give 12 samples of approximately equal size (70–80g). These 12 sub-samples were then assigned at random to either remain raw or to be heated for 1 hr at 40, 45, 50, 55, 60, 65, 70, 75, 80, 87.5, or 95°C.

For Experiment 2 each pair of veal SM muscles was subdivided after thawing to give 16 samples of similar size (50–70g) which were then randomly assigned to be heated at 50, 55 or 60°C for 0.5, 1, 2, 4, or 8 hr or at 80°C for 1 hr. The end pieces remaining from the muscles of the 17 yr old steers were thawed and subdivided into 10 samples of approximately equal size (70–80g) which were assigned to 50, 55, or 60°C for 1, 24, or 48 hr or 80°C for 1 hr. In Experiment 2, all samples, except those cooked at 80°C for 1 hr, were divided into two approximately equal parts, after heating and cooling, one of which was then subjected to further heating at 80°C for 1 hr.

Cooking methods

All samples were wrapped in polyethylene bags and heated, totally immersed, in water baths maintained at the desired temperature (± 0.5°C) for the required time. After heating, each sample was cooled in cold running water for at least 30 min and dried with paper towels before placing in polyethylene bags and storing overnight at 0–1°C. For Experiment 2 (a) samples were weighed both before and after

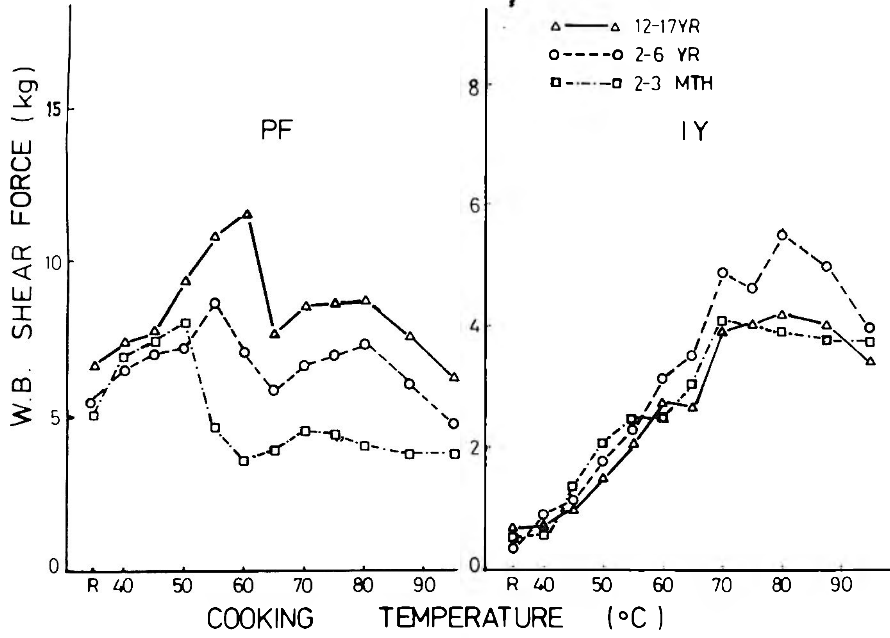

Fig. 1—Warner-Bratzler peak and initial yield shear force values obtained for samples of stretched SM muscles from steers either raw (R) or heated for 1 hr. The LSD's within each age group were 1.48, 1.31 and 1.79 kg respectively for PF values and 1.03, 0.57 and 0.51 kg for IY values.

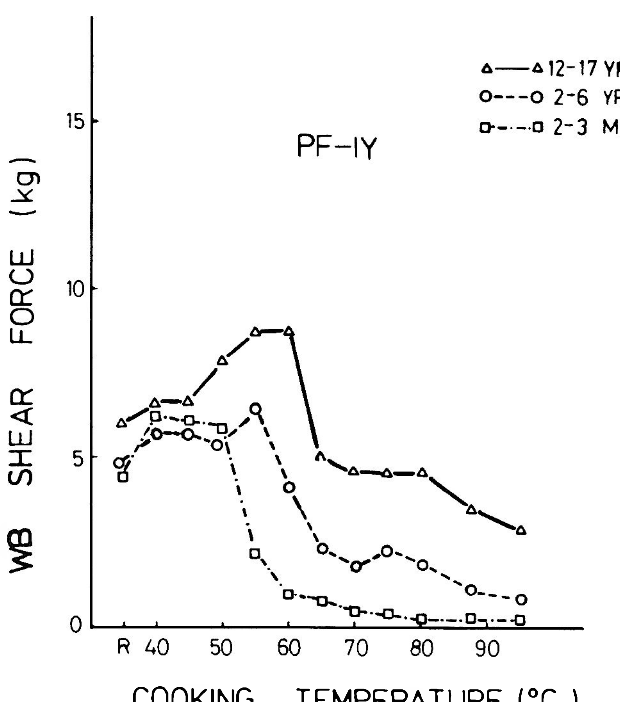

Fig. 2—Warner-Bratzler peak-initial yield force values obtained for samples of stretched SM muscles from steers either raw (R) or heated for 1 hr. The LSDs within each age group were 0.89, 1.17, and 1.15 kg, respectively.

cooking to determine moisture loss and (b) after dividing into two parts the part subjected to a further cook at 80°C for 1 hr was also weighed before and after heating so that total moisture loss after the two periods of heating could be calculated.

Measurement of pH and shear force

A Townson expanded scale pH meter with a Philips C64/1 probe type combined electrode (Philips Scientific & Industrial Equipment, Brisbane, Australia) was used to measure ultimate pH values directly on the muscle samples at room temperature (22°C). Only muscle samples with an ultimate pH value in the range 5.4–5.8 were used.

After overnight storage at 0–1°C, the cooked samples were cut into sub-samples suitable for a modified version of the conventional Warner-Bratzler (WB) shear device (Bouton et al., 1975, 1977). These samples were 2-3 cm long and had a rectangular cross section of 1 cm^2 (2/3 × 1½ cm), with the muscle fibers lying parallel to the long axis of the samples. These sub-samples of the cooked meat were sheared at right angles to the muscle fiber axis. The parameters measured from the shear force deformation curves were (1) peak force (PF) - the maximum force recorded and (2) initial yield force (IY) - the force at which the sample first began to yield, i.e. the first major inflexion on the curve. Peak minus initial yield force (PFIY) values were then calculated.

Statistical methods

Analysis of variance was used to test for significance of treatment effects and to calculate, where appropriate, least significant difference (LSD) values at the $P<0.05$ level.

RESULTS & DISCUSSION

Influence of animal age (Experiment 1)

The WB shear force results obtained for samples from stretched SM muscles (from all three age groups), heated for 1 hr at temperatures in the 40–95°C range, are shown in Fig. 1 and 2. Peak force (PF) values (Fig. 1) were influenced little by animal age until heating temperatures reached 50°C. At temperatures above 50°C the PF values obtained for veal decreased and at 55°C animal age differences between all three age groups became significant ($P<0.001$). For the intermediate age and the oldest age groups, PF values decreased ($P<0.05$) for temperatures above 55°C and 60°C, respectively. At temperatures above 65°C, PF values tended to increase up to 80°C ($P<0.01$) before decreasing again for samples from the two older groups.

Initial yield (IY) force values (Fig. 1) increased steadily with increasing heating temperature up to 70–80°C before declining. Animal age effects were not as consistent as with PF values since the samples from the intermediate age group had the highest values.

Previous interpretations of the WB force-deformation curves (Bouton et al., 1975, 1977, 1981) have indicated that IY values reflected primarily the strength of the myofibrillar structure while PF values represented contributions from both the myofibrillar and connective tissue structures. On this somewhat simplistic interpretation PFIY (peak-initial yield force) values would approximate the connective tissue contribution. The PFIY values (Fig. 2) decreased rapidly with increase in heating tem-

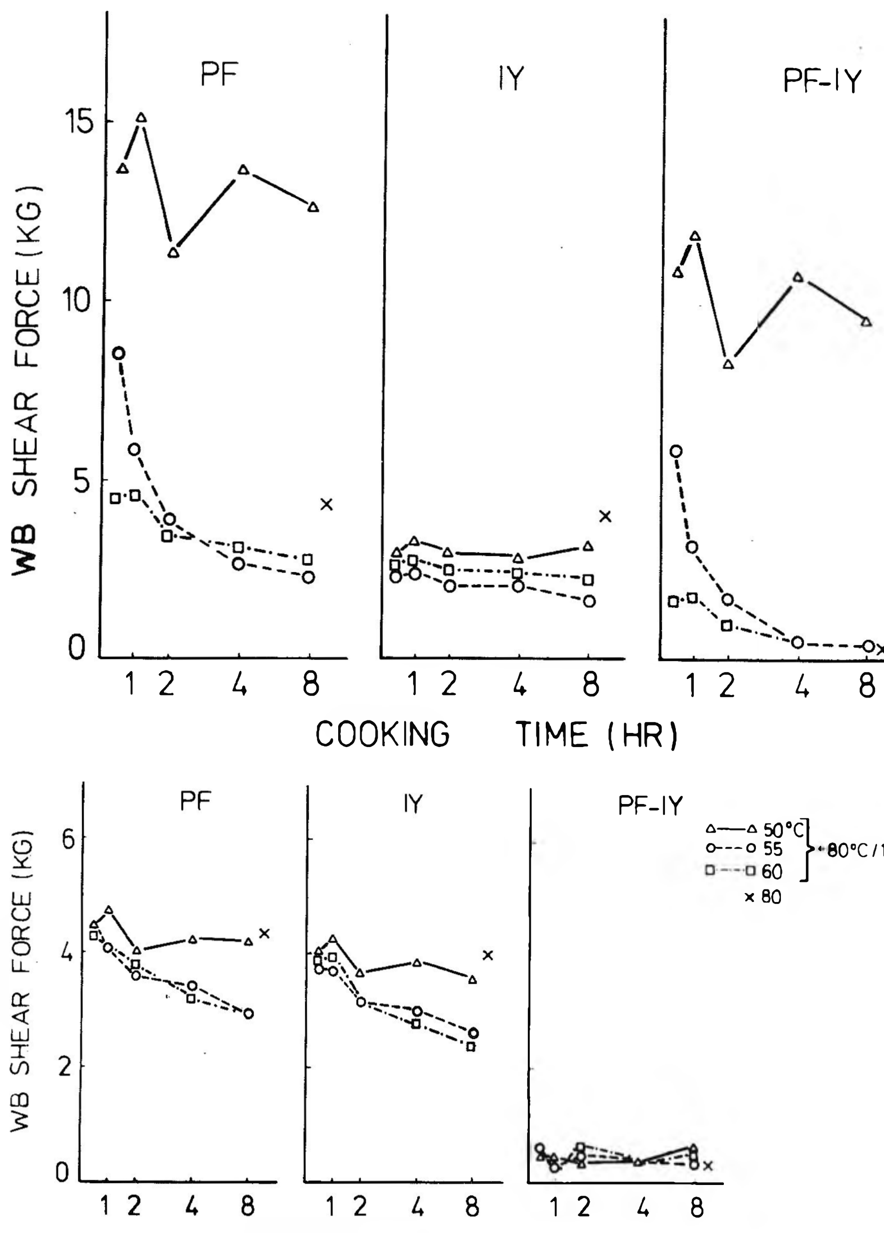

Fig. 3—Warner-Bratzler peak, initial yield and peak-initial yield force values obtained for stretched SM muscle samples from 2-3 month old calves heated at 50°, 55° or 60°C for 0.5, 1, 2, 4 or 8 hr or 80°C for 1 hr. The LSDs for each parameter were 4.44, 1.16, and 3.85 kg at 50°C, 2.65, 0.78, and 2.08 kg for 55°C, and 0.64, 0.42, and 0.75 kg for 60°C.

Fig. 4—Warner-Bratzler peak, initial yield and peak-initial yield force values obtained for stretched SM muscle samples from 2-3 month old calves heated at 80°C for 1 hr after initially heating at 50°, 55° or 60°C for 0.5, 1, 2, 4, or 8 hr. The LSDs for each parameter were 0.67, 0.70, and 0.47 kg, respectively.

perature above 50°C for the veal samples, reaching very low values when 70°C was used. The PFIY values obtained for the older age groups decreased rapidly as temperatures employed were increased above 55°C (for the intermediate age group) and 60°C (for the oldest group). The oldest age group had high PFIY values even after samples had been heated at 95°C for 1 hr.

These results (Fig. 1 and 2) confirmed earlier findings (Bouton et al., 1981) that changes in PF (or PFIY) values obtained after heating in the range 50–65°C (for 1 hr) were related to changes in the connective tissue. These changes in PF values could have been due to heat-induced changes in the stress-strain characteristics of the collagenous connective tissue (Snowden et al., 1977) but measurement of the adhesion between the meat fibers has indicated that at cooking temperatures between 50 and 65°C a loss of adhesive strength occurs (Bouton and Harris, 1981). The WB shear measurements appeared to reflect a real loss of connective tissue strength. The apparent loss of this strength also appeared to occur at different cooking temperatures for muscles of the various age groups.

Comparison with earlier work

The results shown in Fig. 1 and 2 were obtained using a modified version of the WB shear device. Earlier results have been obtained using a conventional WB shear device (Machlik and Draudt, 1963; Draudt et al., 1964; Schmidt et al., 1971; Draudt, 1972; Bouton and Harris, 1972; Bouton et al., 1974) and a MIRINZ tenderometer (Davey and Gilbert, 1974, 1976; Davey and Niederer, 1977). The former group showed that large decreases in shear force values occurred when temperatures in the 50–65°C range were used, while the latter showed a three- to fourfold increase in shear force values with increase in heating temperatures from 40° to 60°C. The apparent dichotomy was explained in some relatively recent work (Bouton et al., 1981) which showed that, using the modified WB shear device, PF values were similar to those obtained with the conventional WB shear device while IY values showed similar results to those obtained using the MIRINZ tenderometer.

Effect of cooking temperature and time (Experiment 2)

These experiments were designed to investigate the effect on shear and other properties of (a) heating meat samples at 50, 55 or 60°C for different times and (b) cooking such samples at 80°C for 1 hr after such treatments.

Muscles from young animals

The effect on WB shear force values of cooking veal muscles at 50, 55 or 60°C for 0.5, 1, 2, 4, or 8 hr, or at 80°C for

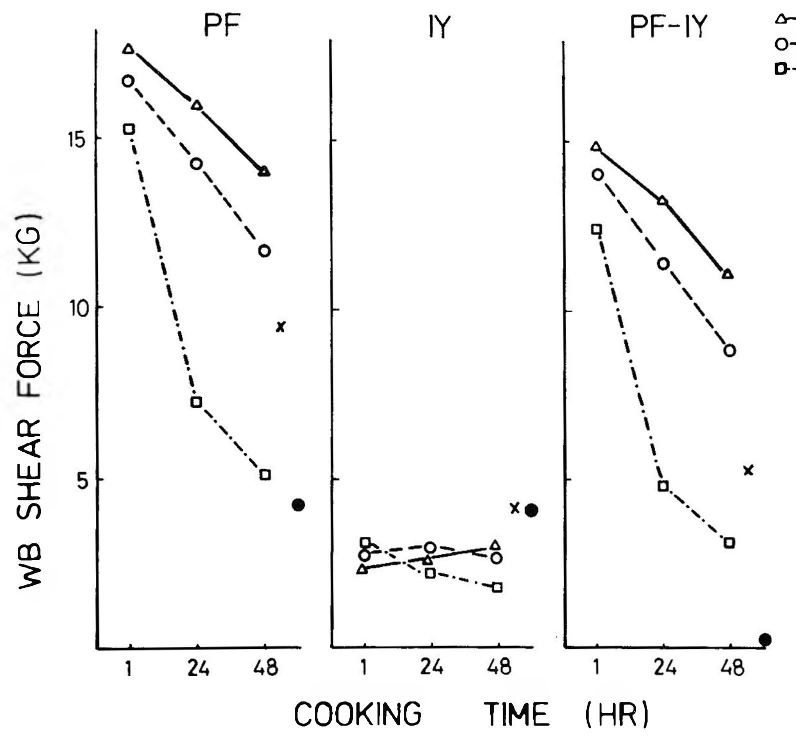

Fig. 5—Warner-Bratzler peak, initial yield and peak-initial yield force values obtained for stretched SM muscle samples from 17 year old steers heated at 50°, 55° or 60°C for 1, 24 or 48 hr or 80°c for 1 hr. The LSDs for each parameter were 3.63, 0.71, and 3.31 kg, respectively.

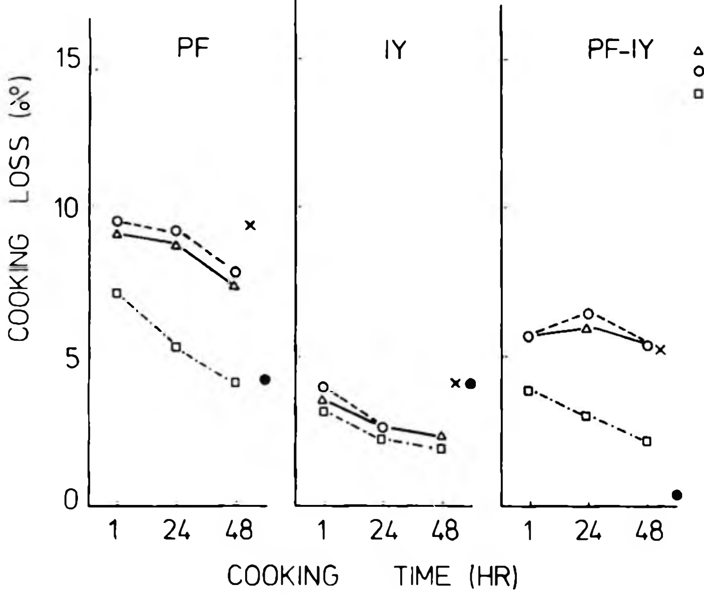

Fig. 6—Warner-Bratzler peak, initial yield and peak-initial yield force values obtained for stretched SM muscle samples from 17 year old steers heated at 80°C for 1 hr after initially heating at 50°, 55° or 60°C for 1, 24 or 48 hr. The LSDs for each parameter were 2.06, 0.64, and 1.72 kg, respectively.

1 hr are shown in Fig. 3. Duration of cooking at 50°C had little effect on PF values but increasing the cooking time at 55°C or 60°C produced significant decreases. The PFIY results were similar and, if the interpretation (Bouton et al., 1975) of the shear force deformation curves is correct, would indicate a dramatic loss in connective tissue strength which was (a) faster at 60°C than at 55°C, (b) almost complete within 4 hr at 55 or 60°C and (c) almost complete in 1 hr at 80°C.

The contrast between the results obtained for the samples heated at 50–60°C for 0.5–8 hr (Fig. 3) and those obtained for these same samples which have been subjected to a further cook at 80°C for 1 hr (Fig. 4), is marked. In the latter case the PF and IY values are nearly identical (i.e. PFIY values are close to zero) within each treatment. Connective tissue strength would be expected to be very low so that both PF and IY values should be indicative of changes in the strength of the myofibrillar structure. The IY (and PF) values decreased with increased initial heating time at 50°, 55° or 60°C (Fig. 4). These results thus indicated that high temperature aging was a factor in any tenderization with extended heating in the 50–60°C region.

Muscles from old steers

In earlier work (Bouton and Harris, 1981) there were indications that cooking times as long as 48 hr might be required to have a significant and large effect on shear force values of muscles from very old animals. The samples from the old steers in this present experiment were thus heated for 1, 24 or 48 hr at 50°, 55° or 60°C as well as for 1 hr at 80°C.

The results obtained are shown in Fig. 5. There was no significant temperature effect for the samples heated for only 1 hr but at 24 or 48 hr the samples heated at 60°C had lower values ($P<0.05$) than those heated at 50° or 55°C. Heating for

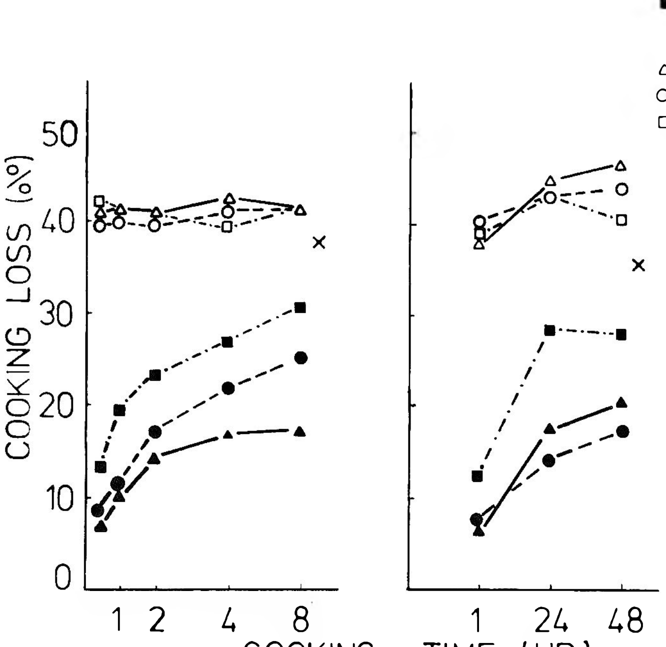

Fig. 7—Percent moisture loss values obtained for stretched SM muscle samples from 2-3 month old calves initially heated at 50°, 55° or 60°C for 0.5–8 hr and from 17 year old steers initially heated at 50, 55 or 60°C for 1, 24 or 48 hr with and without a subsequent heating of 80°C for 1 hr. The LSDs for calves and steers, respectively, were 1.6 and 2.6% for initial cooking losses, 2.0 and 2.1% for total cooking losses.

48 hr decreased PF and PFIY values at all three temperatures but only for 55° and 60°C were the decreases significant. The IY values varied little with cooking temperature (Fig. 5) and only for the 60°C samples was the effect of heating time significant. The IY values at all three temperatures and times were significantly lower than the value obtained for the samples heated at 80°C for 1 hr.

The additional heat treatment at 80°C resulted in a decrease ($P<0.05$) in IY values with increase in heating time for all three initial heating temperatures (Fig. 6). These IY values were less ($P<0.01$) than the value obtained for the samples heated at 80°C for 1 hr - except for the samples heated at 50 or 55°C for 1 hr. Only at 60°C were the PF and PFIY values reduced, i.e. significantly less than the value obtained at 80°C for 1 hr. Even after 48 hr at 60°C the PFIY values were significantly greater than those obtained for veal cooked for 0.5–8 hr (Fig. 6).

Cooking loss measurements

Cooking losses for the veal samples increased (Fig. 7) with both cooking temperature and duration of cooking and reached over 30% in 8 hr at 60°C. This was close to the loss obtained for samples cooked at 80°C for 1 hr. The losses obtained from the samples from the older animals were similar to those obtained for the young animals, even ignoring the prolonged cooking times. Cooking losses increased with time at 50 and 55°C whereas at 60°C there was no difference in losses at 24 and 48 hr. Even after this length of time the losses were not as high as for samples cooked at 80°C for only 1 hr.

The samples initially cooked at 50–60°C were subjected to a further cook at 80°C for 1 hr (Fig. 7) and the total cooking loss for the veal was over 40%. The extent of this total loss was not related to the duration or temperature of cooking. For the samples from the older animals, for which far longer cooking times were used, the total moisture losses increased ($P<0.001$) with cooking time and were ($P<0.01$) greater for 50° and 55°C temperatures than for those initially heated at 60°C.

Obviously, the moisture lost during cooking produces changes in the dimensions of individual samples but at temperatures below 60°C the changes in muscle fiber length are likely to be relatively small compared to the changes in cross sectional area (Hostetler and Landmann, 1968; Bouton et al., 1976). Changes in cross-sectional area would produce similar changes in fiber packing density. In Fig. 3 and 5 the IY results were thus obtained for samples where fibre packing density was likely to be changing with cooking temperature and time whereas the IY results in Fig. 4 and 6 were obtained for samples where cooking losses (and hence fiber packing densities) were relatively constant.

Interpretation of results

Several suggestions have been made about the nature of the changes that occur in meat during cooking. According to one suggestion (Davey and Niederer, 1977), during heating to 65°C meat is tenderized by specific proteolytic attack on the myofibrillar structure (i.e., accelerated aging) and above 70°C there is a destruction or weakening of the collagenous connective tissue. This interpretation does not agree with other published work (Machlik and Draudt, 1963; Tuomy et al., 1963; Draudt et al., 1964; Bramblett et al., 1959; Bramblett and Vail, 1964; Bayne et al., 1969; Laakkonen et al., 1970; Penfield and Meyer, 1975) which suggests that collagen is weakened at temperatures well below 70°C. If prolonged heating in the 50–65°C range acted only via proteolysis of the myofibrillar structure, then the usefulness of such treatments would be limited, since it has been demonstrated (Ratcliff et al., 1977), using a pressure-heat method, that decreasing myofibrillar strength alone does not make all meat tender.

In this work, using stretched muscles from animals of known age the WB peak force values decreased as cooking temperatures increased above 50°C for veal muscles, 55°C for muscles of animals between 2 and 6 years of age and 60°C for muscles of 12–17 yr old steers. This work also showed that WB initial yield force value increased with increase in cooking temperature. In earlier work (Bouton et al., 1981), IY values changed in the same way as shear values obtained using a MIRINZ tenderometer (Davey and Gilbert, 1974). WB peak force values were affected in ways which suggested that they had a component related to connective tissue strength while the IY

values showed (as did the MIRINZ tenderometer) effects suggestive of variations in myofibrillar strength only. The results in this present paper could thus be interpreted as showing that with extended cooking times at temperatures between 55° and 60°C, there were significant decreases in connective tissue toughness, even for muscles of very old animals, along with significant decreases in myofibrillar toughness.

Extended cooking at temperatures close to 60°C thus appeared to tenderize the meat both by accelerated aging (Davey and Niederer, 1977) and by reducing connective tissue strength (Machlik and Draudt, 1963; Draudt et al., 1964; Draudt, 1972; Bouton and Harris, 1972, 1981; Bouton et al., 1974, 1981; Laakkonen et al., 1970; Penfield and Meyer, 1975). The question which must now be asked is whether such a cooking method is economically viable. Cooking losses of about 30% would occur in 48 hr at 60°C and, if a further cooking was required, then total losses as high as 40% might be expected. Dinardo et al. (1984) have recently shown that holding meat at 60°C for up to 4 hr decreased subjectively determined juiciness and flavor without having any significant effect on tenderness. Shear force values, however, showed a small decrease for muscles from the round (Dinardo et al., 1984). The cooking times used by these workers (Dinardo et al., 1984) were considerably shorter than the cooking times used in this present paper. This suggested that much of the gain in increased tenderness with extended cooking could be counterbalanced by significant losses in juiciness and flavor.

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Effect of Calcium Activated Protease (CAF) on Bovine Myofibrils Under Different Conditions of pH and Temperature

MICHAEL G. ZEECE, RICHARD M. ROBSON, MARY LEE LUSBY, and FREDERICK C. PARRISH JR.

ABSTRACT

This study examined the *in vitro* effects of calcium-activated protease (CAF) on bovine myofibrillar proteins and structure under postmortem-like conditions of pH and temperature. Effects usually associated with this enzyme under optimal conditions were reduced as temperature and/or pH were lowered. However, significant activity remained at 15°C and pH 6.5, and some activity was detectable at even lower pH's and temperatures. Effects observed included: solubilization of myofibrillar protein, degradation of the myofibrillar protein titin and others, and an increase in the degree of myofibrillar fragmentation. These results suggest that CAF is able to hydrolyze proteins that are important to structural integrity under conditions mimicking those present in postmortem muscle.

INTRODUCTION

THE POSTMORTEM AGING of meat results in increased meat tenderness and quality. Many factors such as age, sex, and nutritional state of the animal affect the overall acceptability of the meat. However, the increase in tenderness that is associated with postmortem aging probably is due to the action of endogenous proteolytic enzymes (Goll et al., 1983a). Recent reviews have identified the cathepsins B, D, H, and L and two forms of a calcium-activated protease (CAF) as being endogenous to muscle fibers and having a potential role in this process (Dutson, 1983; Goll et al., 1983a). Because of their putative importance to protein turnover *in vivo* and to meat quality, much research is being conducted to better understand the action of these proteases on myofibrillar proteins and structure.

Increase in meat tenderness postmortem is assumed by many investigators to be the result of breaking, fragmenting, or at least weakening of the myofibrillar structure at or near the myofibrillar Z lines (Parrish et al., 1973; Lawrie, 1980, 1983; Robson et al., 1980, 1981, 1984; Goll et al., 1983a). Therefore, the action of a proteolytic enzyme that is effective in postmortem muscle should be to catalyze the hydrolysis of one or more proteins at a structurally important site(s) that results in weakening of the myofibril. Although a number of studies have been done to assess the relative effectiveness of cathepsins B, D, H, and L in hydrolyzing myofibrillar proteins (Bird and Schwartz, 1977; Bird and Carter, 1980), few studies have taken into account the actual chemical and physical environment present in postmortem muscle.

CAF is a thiol endoprotease having a pH optimum of 7.5 (casein) and requires the divalent cation, calcium, for activation. It contains two subunits of approximately 80,000 and 30,000 molecular weight each. CAF has been shown to degrade the myofibrillar/cytoskeletal proteins filamin, titin, C-protein, desmin, tropomyosin, and troponins T and I (Goll et al., 1983b; Zeece et al. 1983). The major form of CAF in muscle cells requires millimolar concentrations of calcium for activation; (Dayton et al., 1975; 1976a,b); however, recent studies have shown that there is another form of the enzyme present in muscle that only requires micromolar concentrations for activation (Goll et al. 1983b). The millimolar form is often called high-calcium CAF, and the micromolar form is often called low-calcium CAF (alternatively they are referred to as calpain II and calpain I respectively, Murachi, 1983). If the millimolar form of the enzyme is allowed to autolyze slightly, then the calcium concentration required for activation is reduced to the micromolar range (Edmunds et al., 1984). Neither form of the enzyme has been shown to have any difference in the hydrolysis of myofibrillar proteins, and little is known about the effectiveness of either form of the enzyme under less than optimal conditions. The goal of this study was to gain a greater understanding of the myofibrillar disassembly process and of the effects that different conditions of pH and temperature exert on the enzyme. In this *in vitro* study, a highly purified form of high-calcium CAF was used. Sufficient calcium concentrations were used throughout the study to examine the combined effects of the high-calcium CAF and any autolyzed versions.

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MATERIALS & METHODS

Myofibril preparation

Myofibrils were prepared from bovine *longissimus* muscle. Approximately 100g muscle was removed within 1 hr after exsanguination. The muscle was trimmed free of excess fat and connective tissue and passed through a precooled meat grinder. Myofibrils were prepared from 50 g ground muscle according to the method of Goll et al. (1974).

CAF preparation

CAF was extracted from 2–3 kg bovine cardiac muscle by the procedure described by Dayton et al. (1976a) for porcine skeletal muscle, with the exception that fractionation at pH 6.5 was eliminated. This procedure yielded a crude CAF preparation, which was chromatographed by the method of Wolfe et al. (1985), which included affinity purification using the peptide inhibitor of CAF (antipain) bound to Sepharose 4B. The purification procedure yielded 20–30 mg highly homogenous CAF with a specific activity of 200–300 units/mg (1 unit = 0.1 absorbance reading at 278 nm in the TCA supernatant after 30 min incubation at pH 7.5).

CAF assay with casein

CAF was assayed against casein (purified powder grade, Sigma) by the method of Dayton et al. (1975) with the exception that imidazole-HCl, pH 7.5, was used in place of Tris-acetate. Because imidazole may bind calcium under some conditions, parallel assays were run in Tris-acetate and imidazole-HCl at pH 7.5. It was found that (results not shown) there was no difference in enzyme activity when calcium was at 5.0 mM. The casein reaction mixture contained the following: 5 mg/mL casein, 100 mM imidazole-HCl, pH 7.5, 100 mM KCl, 5.0 mM 2-mercaptoethanol (MCE), 5.1 mM $CaCl_2$, 0.1 mM EDTA, 0.1 mM ethylenediaminetetraacetic acid (EDTA) and 0.1 mM NaN_3.

CAF assay with agarose plate

To rapidly assay CAF activity in a large number of fractions resulting from chromatography, a new "agarose plate method" was devised, incorporating the casein reaction mixture into 1% agarose. The steps in the procedure were: (1) Heating the casein reaction mix-

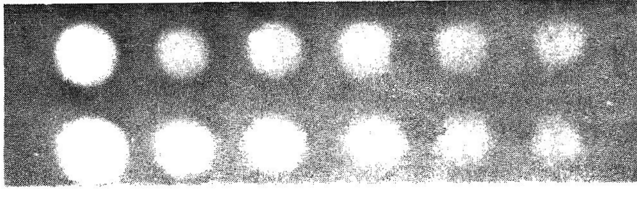

Fig. 1 — Detection of CAF activity by the casein agarose plate method.

Ten μL purified CAF solution containing 10, 2.0, 1.0, 0.2, 0.1, and 0.05 μg protein, respectively, were spotted in duplicate rows.

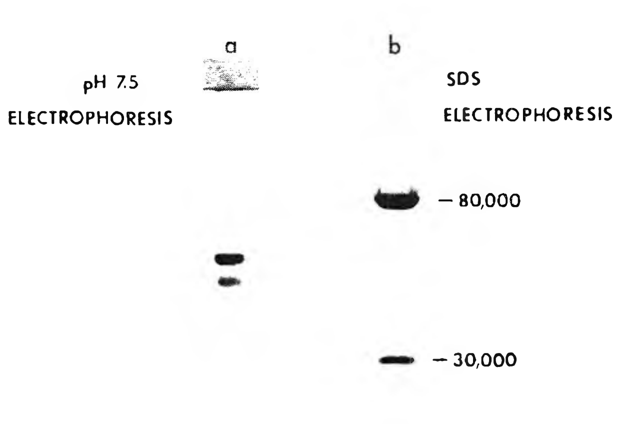

Fig. 2 — Polyacrylamide gel electrophoresis of purified CAF under nondenaturing and denaturing conditions. Purified CAF (20 μg) was electrophoresed under nondenaturing conditions at pH 7.5 on a seven and one-half percent acrylamide gel (a) Purified CAF (28 μg) also was electrophoresed in the presence of SDS on a 10% acrylamide gel (b).

ture used for the tube assay (minus MCE) to 80° C, cooling to 4° C, centrifuging at 10,000 × g_{max} for 10 min, and storing at 0–4° C. (2) Preparing the plates by adding 1.0g LE agarose low-temperature gelling agarose (FMC Corporation, Rockland, ME) to 100 mL reaction mixture from step one and heating in a boiling water bath to 80° C to melt the agarose. Upon cooling to 50° C, MCE was added and the mixture was poured onto the hydrophilic side of gelbond (FMC Corporation) sheets. The mixture rapidly solidified and could be stored for 1 wk at 0–4° C if protected from dehydration. (3) For the detecting of CAF activity, 10 μL aliquots of enzyme fractions were placed at regular intervals (approximately 1.5 cm) along the plate. (4) Incubating the plate for 2 hr at 25° C in a covered container and then staining with a solution of 0.05% (w/v) amido black, 10% acetic acid for 15 min. The plate was washed with 50% methanol, 5% acetic acid for 15 min, and then with several changes of 100% methanol to complete destaining and dehydration. The plate was then allowed to air dry. Proteolytic activity appeared on the plate as clear circles against a blue background. The total time of the assay was approximately two and one half hours.

CAF assay with myofibrils

The myofibrils were assayed under the following conditions: 2.0 mg/mL myofibrils, 100 mM KCl, 50 mM imidazole-HCl of desired pH, 0.1 mM EDTA, 5.1 mM $CaCl_2$, 5 mM MCE. The enzyme-to-substrate ratio was kept at 1:100 (w/w). The pH values tested were 7.5, 6.5, and 5.5 (pH values of 7.0 and 6.0 were also included in the study, but have been omitted from the results for conciseness). At each pH, temperatures of 25°, 15°, and 5° C were tested. The following controls were included: (1) 5 mM EDTA-no enzyme (other reaction conditions the same), (2) 5 mM Ca^{+2}-no enzyme (other reaction conditions the same), and (3) 5 mM EDTA-plus enzyme (other reaction conditions the same). The tubes were preincubated at the desired pH and temperature for 10 min. The reaction was started by the addition of enzyme or by an equal aliquot containing no enzyme in the controls without enzyme. The incubation was run for up to 30 min and stopped by addition of enough concentrated EDTA to bring the final EDTA concentration to 10 mM. The suspension was centrifuged at 50,000 × g_{max} for 15 min at the temperature used in the incubation. The activity of the enzyme was measured as the amount of protein present in the supernatant. For each time point tested, 0.1 mL of supernatant was diluted to 1.0 mL with water and assayed by the Folin-Lowry method (Lowry et al., 1951).

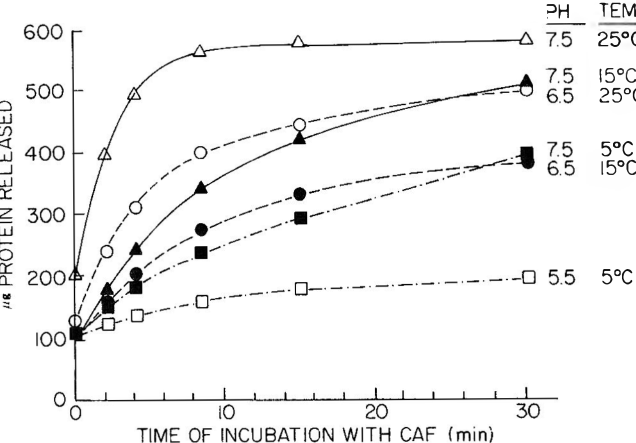

Fig. 3 — Protein released from myofibrils after incubation with purified CAF. Bovine skeletal myofibrils were incubated with purified CAF. Incubations are indicated as follows: pH 7.5, 25° C (△—△); pH 7.5, 15° C (▲—▲); pH 7.5, 5° C (■–·–■); incubation at pH 6.5, 25° C (○– –○); pH 6.5, 15°C (●– –●); incubation at pH 5.5, 5° C (□–·–□).

Degree of myofibril fragmentation

Bovine skeletal myofibrils were incubated with CAF as described above except that the samples were not centrifuged at the end of the incubation time. Instead, they were diluted with 1.0 mL 100 mM KCl and subjected to a brief (10 sec) homogenization in a Virtis homogenizer at a setting of 15,000 rpm. The myofibrils then were examined with a phase-contrast light microscope to determine the effects on structure and degree of fragmentation. The degree of fragmentation was estimated by counting number of sarcomeres per myofibril fragment in at least 10 fields. At least 30 total myofibrils were examined for each sampling pH and temperature. The total number of sarcomeres was divided by the total number of myofibrils counted to obtain the mean number of sarcomeres per myofibril for each incubation condition. The means for the control and CAF-treated samples were analyzed by the students t test.

SDS-polyacrylamide gel electrophoresis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli (1970) with slab gels measuring 110 mm × 160 mm × 1.1 mm. The slab gel consisted of a 5% stacking gel layered over a 10% separating gel. Electrophoresis was performed at a setting of 10 mAmps for 4 hr. Staining was done overnight in a solution of 0.1% Coomassie blue R250 (Sigma Chemical Company), 10% acetic acid, 50% methanol. The gels were destained by diffusion with gentle shaking in a solution of 7.5% acetic acid, 5% methanol.

Electrophoresis in nondenaturing buffers

Electrophoresis in a nondenaturing buffer system was performed in slab or disc gels as described by Dayton et al. (1976a). The slabs were 110 mm × 160 mm × 1.1 mm, and the gel consisted of a 4% stacking gel, pH 5.5, layered over a 7.5 or 10% separating gel, pH 7.5. Samples were applied directly to the gel after incorporation of sucrose to 25% (w/v) final concentration and electrophoresed at 20 mAmps for 4 hr or until the dye front was within 1 cm of the bottom

of the gel. The gels were then stained with 0.1% Coomassie blue as described.

RESULTS & DISCUSSION

BECAUSE CHROMATOGRAPHIC PURIFICATION of CAF requires the rapid assay for activity in a large number of fractions, a casein agarose plate method was developed as described in the Materials and Methods. Figure 1 shows a plate incubated with purified CAF to illustrate the sensitivity of the method. As little as 0.2 μg of purified CAF could be reliably detected. In Fig. 1, two identical rows of CAF dilutions were spotted to also show the reproducibility of the method.

The purified CAF used in this study was examined by several electrophoretic methods. Figure 2a and b show PAGE results with purified CAF under both denaturing and nondenaturing conditions. When the enzyme was electrophoresed under nondenaturing conditions at pH 7.5 on a 7.5% acrylamide gel, it consisted of one major band, with a lighter band migrating just below it, and some very faint bands near the bottom of the gel (Fig. 2a). This pattern is similar to that shown for highly purified CAF in Dayton et al. (1976a). When purified CAF was electrophoresed under denaturing conditions in the presence of SDS on a 10% acrylamide gel, it showed two major bands with molecular weights of about 80,00 and 30,000 daltons each (Fig. 2b).

Two-dimensional electrophoresis (nondenaturing at pH 7.5, followed by SDS-PAGE) was also employed to examine the subunit composition of the major and minor bands observed in the nondenaturing system (results not shown). It was found that the major, slow-moving band in Fig. 2a was composed of 80,000- and 30,000-dalton-components and that the faster-migrating band contained 80,000- and 18,000-dalton components. These results were in good agreement with studies on skeletal muscle CAF by other investigators (Dayton et al., 1976; Goll et al., 1983b) and demonstrated the high purity of the enzyme used in this study.

Myofibrils treated with CAF showed a release of soluble protein, which was dependent on pH and temperature (Fig. 3). A small amount of soluble protein was evident in all samples, even in controls and is represented in the 0-time values. Incubation of myofibrils with CAF at pH 7.5 and 25° C (optimal conditions) exhibited a rapid release of soluble protein. When the temperature of incubation was decreased to 15° C, a substantial release of protein was observed, and at the end of 30 min was nearly that observed for the 25° C incubation. Decreasing the temperature of incubation to 5° C (pH 7.5) greatly reduced the amount of solubilized protein but, did not completely inhibit the enzyme. There was a slow but continuous release of protein under these conditions.

When the incubation was conducted at pH 6.5 at 25° C (Fig. 3) differences were observed in the amount and rate of protein solubilized in comparison with results obtained at pH 7.5 (25° C). The rate of release at pH 6.5 was lower than that obtained at pH 7.5, but substantial solubilization had occurred after 30 min of incubation. Lowering the temperature of incubation to 15° C (pH 6.5) reduced both the initial rate and total amount of protein released. When incubations were conducted at pH 5.5 (5° C), little or no increase in release of soluble protein was detected.

The results concerning release of solubilized protein from myofibrils by CAF presented by Suzuki et al. (1978a,b) are similar to the results presented in this study. However, the work done by those investigators was done with crude enzyme preparations that may have contained other enzymes. This made it difficult to confirm in their study that the observed effects actually resulted from the action of CAF. The work presented herein with purified CAF enzyme demonstrated that CAF is effective in releasing soluble protein from myofibrils *in vitro* and that it is effective over a fairly wide range of pH and temperature conditions.

CAF also was examined systematically with respect to its ability to hydrolyze myofibrillar proteins under different con-

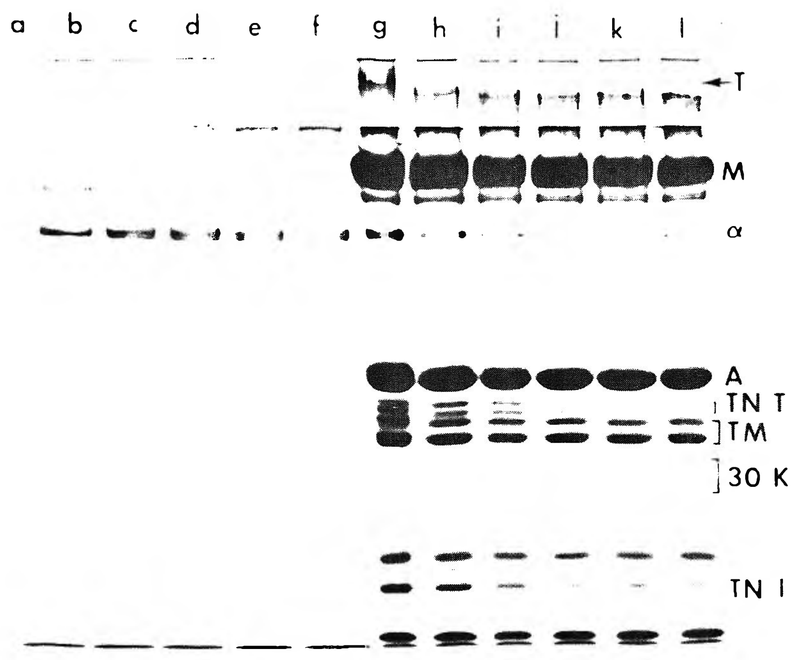

Fig. 4 — Ten percent SDS-PAGE slab gel, of myofibrils incubated with purified CAF at 25° C and pH 7.5. Bovine skeletal myofibrils were incubated with purified CAF. Lanes a through f were loaded with 50 μL of supernatant fractions corresponding to 0, 2, 4, 8, 15, and 30 min of incubation, respectively. Lanes g through l were loaded with 48 μg of sediment fractions from 0, 2, 4, 8, 15 and 30 min of incubation, respectively. Lanes a and g (0 min) correspond to EDTA control supernatant and sediment fractions, respectively. Labels at the right edge of the gel are as follows: T, titin (note that the position of titin marked is that in the 0-time sediment); M, myosin heavy chains; α, alpha-actinin; A, actin; TN T, a small bracket indicates troponin T isomers; TM, a bracket indicates beta and alpha tropomyosin subunits; 30 K, a bracket indicates the position of a trio of bands of approximately 30,000 daltons, TN I, troponin I.

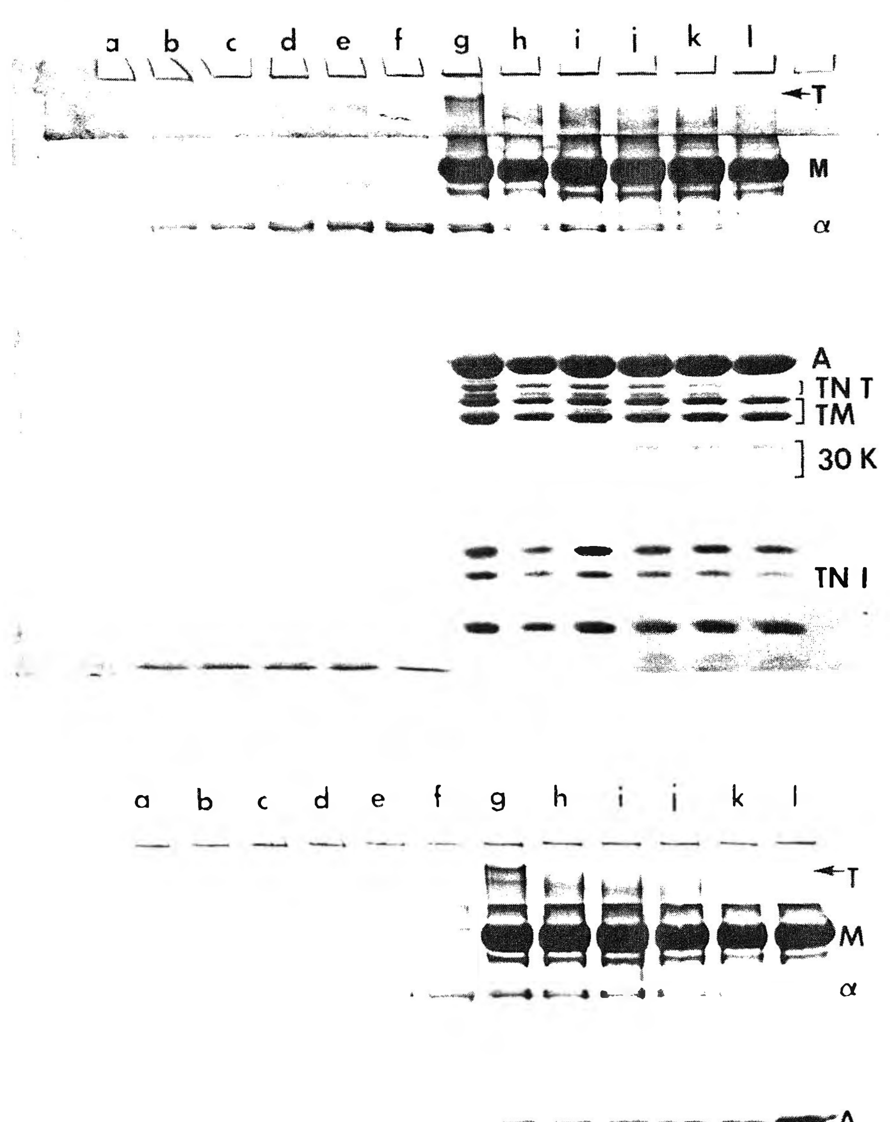

Fig. 5 — Ten percent SDS-PAGE slab gel, of myofibrils incubated with purified CAF at 25° C and pH 6.5. Bovine skeletal myofibrils were incubated with purified CAF. Sample lanes a-f and g-l were loaded as described in Fig. 4. Labels at the right edge of the gel are as described in the legend to Fig. 4.

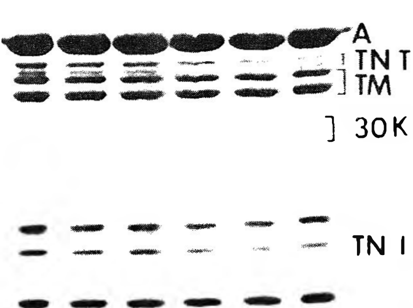

Fig. 6 — Ten percent SDS-PAGE slab gel, of myofibrils incubated with purified CAF at 15° C and pH 6.5. Bovine skeletal myofibrils were incubated with purified CAF. Sample lanes a-f and g-l were loaded as described in Fig. 4. Labels at the right edge of the gel are as described in the legend to Fig. 4.

ditions of pH and temperature by SDS-PAGE (Fig. 4–7). In these experiments, myofibrils were incubated with CAF, centrifuged to separate sediment and supernatant fractions, and then examined by electrophoresis. The effect of incubation of myofibrils with CAF at 25° C and pH 7.5 is shown in Fig. 4. Examination of the supernatant fractions, starting with 2 min of incubation (Lane b), shows that a number of proteins were solubilized and released from the myofibrils by CAF. There were small amounts of several unidentified proteins with molecular weights greater than myosin heavy chains (about 200,000 daltons) and one just below it that were rapidly released. The major protein which migrated just ahead of the myosin heavy chains, may have been M protein (about 170,000 daltons), but this was not shown conclusively. This band appeared in the supernatants of almost all samples (Fig. 4–7), including the controls. The major constituent released (Lanes b-f) appeared to be intact alpha-actinin. In the sediment fractions, there was a decrease in the intensity of the titin band with increased time of incubation. Titin's localization in the electrophoresis stacking gel was identified by using antibody specific for this protein in an immunoblot procedure (results not shown). Putative titin breakdown products, which migrated progressively just faster than titin (cf, lanes h to l with g), also appeared. These results on titin degradation in myofibrils treated with purified CAF correlate well with the observed early loss of titin in whole muscle stored postmortem (Lusby et al., 1983; Robson and Huiatt, 1983) and suggest that CAF may be involved in its postmortem degradation. The identity of the small amount of protein that migrated slightly slower than titin in these samples is unknown. The alpha-actinin band decreased in amount

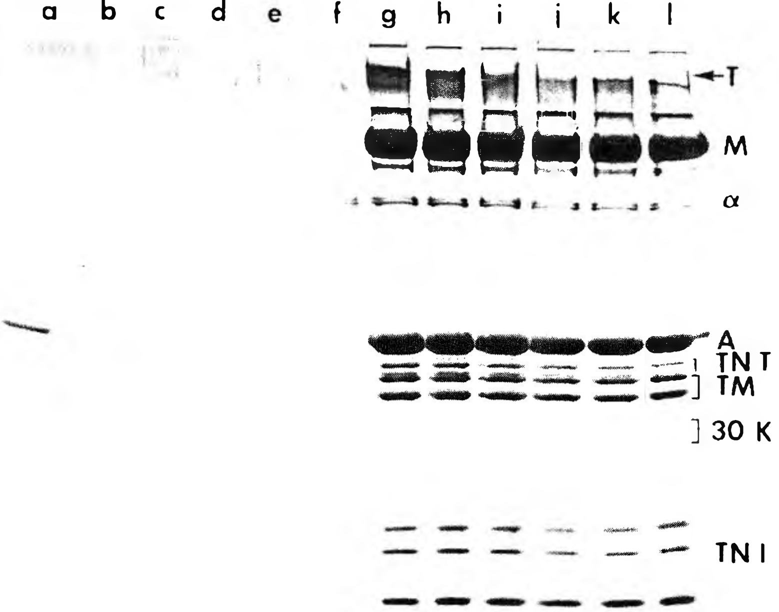

Fig. 7 — Ten percent SDS-PAGE slab gel, of myofibrils incubated with purified CAF at 5° C and pH 7.5. Bovine skeletal myofibrils were incubated with purified CAF. Sample lanes a-f and g-l were loaded as described in Fig. 4. Labels at the right edge of the gel are as described in the legend to Fig. 4.

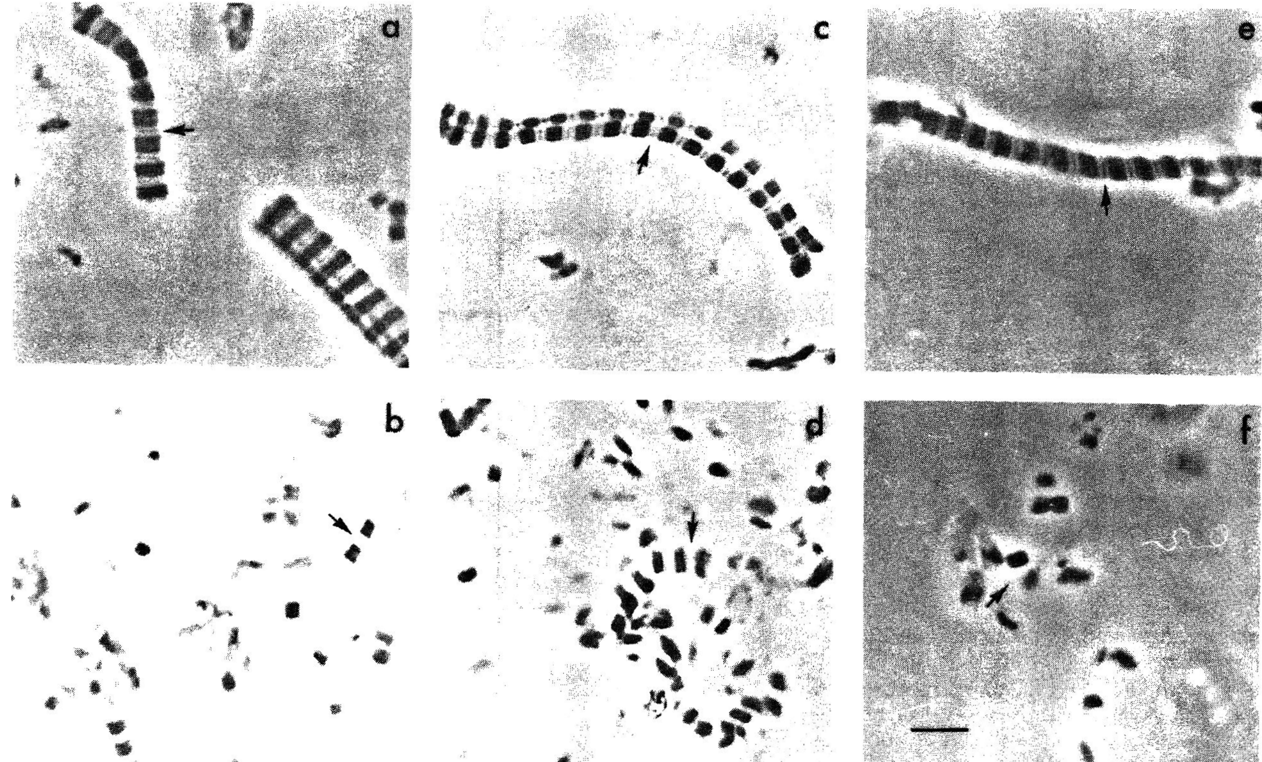

Fig. 8 — Phase contrast micrographs of myofibrils after incubation with purified CAF and a brief homogenization. Assay conditions: Bovine skeletal myofibrils were incubated with purified CAF. Micrographs a, c, and e represent 0 time (EDTA-no enzyme-control), incubations, at pH 7.0, (25° C), pH 6.0, (25° C), and pH 6.5, (15° C), respectively. Micrographs b, d, and f represent 30 min incubations with CAF at pH 7.0, (25° C), pH 6.0 (25° C) and pH 6.5, (15° C), respectively. The arrow in each indicates the position of a Z line or the position from which the Z line has been removed. The bar in micrograph f represents 5 μm. Magnification was ×2000 in all figures.

in the sediments during the incubation. Troponin T isomers were degraded substantially by 4 min (lane i) of incubation time. Tropomyosin subunits were only slightly decreased in amount after 30 min. A trio of bands at about 30,000 daltons appeared in the sediment after only 2 min of incubation (lane h). Their molecular weights determined from the electrophoresis gel are 31,500, 30,000 and 29,000 daltons. It has been proposed that these three bands are breakdown products of troponin T (Dayton et al., 1975; Olson et al., 1976; Goll et al., 1983a) and that their appearence is correlated with tenderness (McBride and Parrish 1977; Parrish et al., 1981). Troponin I was slowly but progressively degraded with increased time of incubation.

The effect of incubating myofibrils with CAF at 25° C and pH 6.5 is shown in Fig. 5. The lanes (a-f) of the supernatants show that somewhat less protein was released from the my-

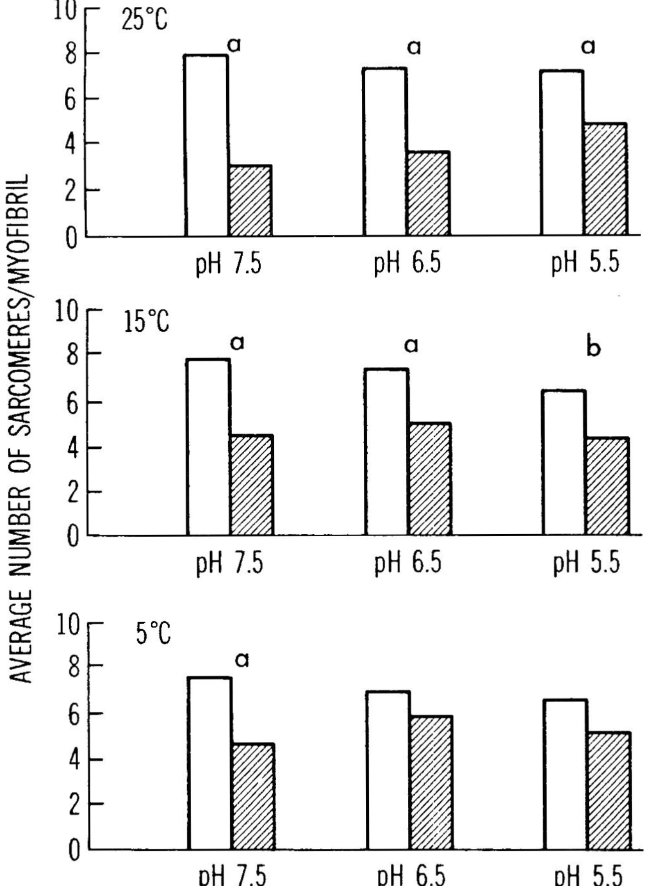

Fig. 9 — Degree of myofibril fragmentation after incubation with CAF at selected pH values and temperatures and a brief homogenization. Myofibrils were incubated with purified CAF at 25°, 15°, or 5° C and pH 7.5, 6.5, or 5.5 for 30 min and then subjected to a brief 10 sec homogenization. The average number of sarcomeres per myofibril was calculated and is shown as open vertical bars for the 0-min samples and as shaded vertical bars for the 30-min CAF-treated samples. The letter (a) indicates a significant difference in the means of control and CAF-treated samples at the .01 level as determined by the students t test. The letter (b) indicates significance at the 0.1 level.

ofibrils than in the experiments done at higher pH (cf., Fig. 4). The supernatants contain some unidentified high-molecular weight-protein, which appears after 4 to 8 min of incubation, migrating with a molecular weight greater than myosin heavy chains. Alpha-actinin appeared to be the major constituent in the solubilized protein. In the lanes (g-1) run on the sediments, the titin band decreased in intensity and migrated as putative breakdown products with increased incubation time. Troponin T and tropomyosin seem to be more slowly degraded than at pH 7.5. The trio of bands (31,500, 30,000, and 29,000 daltons) appeared after 8 to 15 min incubation. Troponin I was only partly degraded after 30 min incubation.

Figure 6 shows the electrophoretic results obtained on supernatants and sediments from myofibrils incubated with CAF at 15° C and pH 6.5. The lanes (a-f) of the supernatants show that somewhat less protein was solubilized than at the higher pH values. The unidentified high-molecular-weight proteins observed at higher pH and temperature values (Fig. 4 and 5) appeared after about 8 to 15 min incubation. The major single constituent in the released protein fraction was alpha-actinin. The lanes (g-1) of the sediments show that titin was degraded to lower-molecular-weight species and that the alpha-actinin band decreased in amount with increase in time of digestion. Troponin T was only partly degraded during the 30 min incubation, and tropomyosin and troponin I were degraded only slightly. The trio of bands (31,500, 30,000, and 29,000 daltons) appeared after about 8 to 15 min incubation.

Figure 7 shows the results for the digestion at 5° C and pH 7.5. The lanes (a-f) run with the supernatant samples show that only a small amount of protein was solubilized. There were some unidentified high-molecular-weight proteins and alpha-actinin. The latter protein was quite evident after 8 to 15 min incubation. The lanes (g-1) of the sediments show that titin was degraded during the 30 min incubation. As expected, there was a small decrease in the amount of the alpha-actinin band in the sediments with increased incubation time. A small decrease in troponins T and I occurred as well. The trio of degradation bands (31,500, 30,000, and 29,000 daltons) was present in minor amounts after 15 to 30 min incubation.

In general, the extent of changes observed in results obtained by SDS-PAGE analysis of samples from CAF-treated myofibrils (Fig. 4–7) reflected the same time course, pH, and temperature effects seen in the studies on protein released (Fig. 3). Decrease in either pH from 7.5 or in temperature from 25° C reduced the observed changes. However, significant changes were still seen during digestions done at 15° C and pH 6.0–6.5 or at 5° C and pH 7.5.

CAF also was examined systematically with respect to its effectiveness in altering the structural integrity of the myofibril under different conditions of pH and temperature. The structures of myofibrils that had been incubated with CAF for 30 min at pH 7.0 (25° C), pH 6.0 (25° C), and pH 6.5 (15° C) and then homogenized were compared with the EDTA control myofibrils for these incubations, respectively (Fig. 8). The myofibril fragments in the controls were fairly long and contained intact Z-lines. By the end of 30 min incubation with CAF (Fig. 8b, d, and f), there was a marked increase in degree of fragmentation. The myofibrils also occasionally exhibited a rather distorted or stretched appearance such as that shown in Fig. 8d. Myofibril fragmentation resulting from a loss of structural integrity was also observed by Olson et al. (1976) and it has been suggested as a measure of tenderness (Olson and Parrish, 1977). These results lend support to the idea that this enzyme is involved in myofibrillar disassembly *in vivo* and under postmortem-like conditions.

The extent of fragmentation of treated myofibrils was difficult to accurately assess because even untreated, freshly prepared myofibrils contained a population in which the number of sarcomeres per myofibril varied from 1 to 20 or more. Therefore, the number of sarcomers in individual myofibrils were counted in an attempt to determine more objectively if CAF treatment caused significant fragmentation. The results in Fig. 9 show the average number of sarcomeres per myofibril for the EDTA-no enzyme-control and 30-min samples incubated at 25°, 15°, or 5° C and at pH 7.5, 6.5, or 5.5, respectively. In general, it was found that a decrease in the average number of sarcomeres per myofibril coincided with alterations observed with electrophoresis (not all corresponding electrophoresis gels shown). When myofibrils were incubated at 25° C, there was little change in the controls at pH 7.5, 6.5, or 5.5. The sample incubated with CAF for 30 min at pH 7.5 showed a sharp decrease (from about 8 in the control to 3.5) in the average number of sarcomeres per myofibril. A similar difference was observed between control and treated myofibrils at pH 6.5. At pH 5.5, a small decrease in the average number of sarcomeres per myofibril in the treated sample occurred compared with its control. The average number of sarcomeres per myofibril in the CAF-treated samples increased gradually with decreasing incubation pH at 25° C. When myofibrils were incubated with CAF at 15° C and pH 7.5, 6.5, or 5.5, and then homogenized, there was a smaller decrease in the average number of sarcomeres per myofibril for each of the treated samples compared with its control than was obtained in the samples incubated at 25° C. When myofibrils were incubated with CAF at 5° C and pH 7.5, 6.5, or 5.5 and then homogenized, the effect of the CAF was reduced yet further, although

the treated samples still had fewer sarcomeres per myofibril than the respective controls.

The results (Fig. 8 and 9) showed that CAF can cause an increase in loss of Z-line integrity and in degree of myofibril fragmentation *in vitro* within a fairly wide range of pH and temperature conditions. This evidence adds further support to the suggestion that CAF has a principal role in the postmortem tenderization process (Goll et al., 1983a; Olson et al., 1976). Additional studies will be required to answer important questions such as the Ca^{2+} concentration required for activation of CAF postmortem and how the CAF/CAF inhibitor interaction is controlled and modified under postmortem conditions.

CONCLUSIONS

THE RESULTS of these *in vitro* studies with the high-calcium form of CAF indicate that this enzyme can cause a number of alterations in myofibrillar proteins and structure within a range of pH and temperature conditions similar to those present for at least several hours in muscle cells after death. These alterations result in a loss of structural integrity of the myofibrils and an increase in the degree of fragmentation. Thus, this study provides additional support for CAF's involvement in textural and quality changes that occur in postmortem muscle.

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Characteristics of Color in Raw, Baked and Smoked Wild and Pen-Reared Atlantic Salmon

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ABSTRACT

Color of wild, astaxanthin pigmented, and farmed, canthaxanthin pigmented, Atlantic salmon (*Salmo salar*) was evaluated by spectroscopy and visual sensory analyses. A more yellow hue in farmed salmon color compared with wild salmon was found in acetone extracts of raw salmon flesh and by visual sensory analyses of raw, baked and smoked salmon flesh. With instrumental color analysis directly on raw flesh, no significant differences in color between wild and farmed salmon were found. The redness and hue in raw and baked salmon flesh and the redness in smoked salmon were correlated to the pigment concentration in raw salmon. The redness and hue in processed salmon were predictable from the redness and hue of raw flesh.

INTRODUCTION

REDNESS of flesh is one important quality criterion in salmon. The characteristics of salmon-red color should be maintained also when the fish is processed and ready to consume. Prediction of product color based on the raw salmon color would be a real advantage in the salmon farming industry thus enabling it to satisfy the markets' varying demands for pigmentation.

Astaxanthin is the dominant carotenoid pigment in the flesh of wild salmon (Khare *et al.*, 1973; Schiedt *et al.*, 1981). In salmon farming both astaxanthin obtained from shrimp processing offal and synthetic canthaxanthin are utilized as pigments. Feeding salmon synthetic astaxanthin still is in the experimental stage (Storebakken *et al.*, 1985).

Spectral characteristics have frequently been used to evaluate the pigmentation of salmonids. Most studies have used the reflectance spectra of raw or processed muscle tissue and further transformation into CIE XYZ tristimulus values and various uniform color systems (Hunter, 1975). In these systems, salmon color may be described by the parameters L, a and b where L represents lightness, a redness and b yellowness of the sample. A ratio between a and b gives an expression of the hue of the color. Schmidt and Idler (1958) and Saito (1969) found redness (+a) obtained in a Gardner automatic colorimeter, to be proportional to the carotenoid content of sockeye (*Oncorhynchus nerka*) and Atlantic salmon (*Salmo salar*), respectively. More recently, Choubert (1982) found a positive correlation between the excitation purity (P_e) of the CIE XYZ system, a measure of color saturation, and the canthaxanthin concentration in farmed rainbow trout (*Salmo gairdneri*). Schmidt and Cuthbert (1969) reported a positive correlation between the ratio of the reflectance at 650 nm and 510 nm and the ratio of Hunter a and b values of raw coho salmon (*O. kisutch*). This reflectance ratio has later been utilized in sorting salmon (Francis and Clydesdale, 1975).

Some discrepancies exist in the literature concerning the interpretation of the color parameters as far as perceived color is concerned. Schmidt and Idler (1958) reported Hunter +a values to be a good measure of visual color preference in raw sockeye salmon. Further, they found that Hunter +a values were suitable for measuring processed salmon color and that the color could best be predicted from the Hunter a/b ratio of the raw flesh. However, Little *et al.* (1979) reported that multiple regression analyses had to be applied to make color parameters in various color systems match the visual evaluation of raw rainbow trout. Among the systems evaluated, they found only CIE Z and P_e to be directly correlated to the visual score. Pigment concentration could not be used to predict color of the muscle tissue.

The purpose of the present experiments was to study the relationships between instrumental color parameters of raw, baked and smoked wild and farmed Atlantic salmon and carotenoid concentration. A further aim was to determine how variations in pigment type and concentration influence the appearance of raw and processed salmon flesh.

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MATERIALS & METHODS

Fish material

The study was carried out on 39 Atlantic salmon. Eighteen salmon were caught in the North Sea (average weight $\bar{x}=5.2$ kg, standard deviation $s=0.5$), 6 were caught in the river Driva at Sunndalsøra, Norway ($\bar{x}=6.7$ kg, $s=1.9$), and 15 were raised in net-pens ($\bar{x}=4.8$ kg, $s=0.6$). During analyses, no differentiation could be made between the two groups of wild fish, and they were therefore treated as one group during the experiments. The farmed salmon which had been kept in the sea for 2 yr, were fed a moist diet consisting of fish silage (50%), fish meal (10%) and a binder meal (40%). The binder meal contained canthaxanthin (Carophyll Red. F. Hoffmann-La Roche & Co. Ltd., Basle, Switzerland) at a concentration of 50 mg/kg. After slaughtering, the salmon were stored frozen (−20°C) for 2–5 wk prior to evaluation. Based on carotenoid concentration, 16 individuals were selected for processing and color analyses of salmon flesh.

Chemical analyses

Carotenoid analyses. The carotenoid concentration of the tail of each raw salmon (39 individuals) was determined. Before analyses, the tails were cut at the adipose fin, freed from skin, bone and fat tissue and minced with a knife. Twenty grams of homogenate were extracted in 3 × 50 mL acetone during a 24-hr period. Extractions were performed at 4°C in the dark and in an N_2-atmosphere. Carotenoid concentration in each salmon was calculated from extinctions of the extracts read at λ_{max}. An average extinction coefficient $E^{1\%}_{1cm} = 1900$ was used for both astaxanthin and canthaxanthin (Foss *et al.*, 1984).

Acetone extracts from salmon were pooled into three samples: (1) extracts from salmon caught in the river; (2) extracts from sea salmon; and (3) extracts from farmed salmon. The pooled extracts were subjected to analysis for identification of type of carotenoid by HPLC as described by Storebakken *et al.* (1985).

Proximate analyses. Homogenates of the tail samples from the 16 fishes selected for processing and color analyses were analyzed for dry matter (105°C, overnight), ash (550°C, overnight), crude protein (Kjeldahl) and lipid (Folch *et al.*, 1957) content.

Salmon sectioning and processing conditions

The 16 salmon selected for processing and color analyses were sectioned according to the following procedure; two 1.5 cm thick slices were cut anterior to the adipose fin and utilized for further studies of raw muscle. The section from the head posterior to the dorsal fin was filleted and subjected to smoking. The rest of the carcass was utilized for baking.

Baked salmon were prepared by heating steaks packed in aluminium foil in a cooking cabinet to a core temperature of 70°C. Smoked salmon were prepared by salting fillets in brine (10 kg NaCl and 3 kg sucrose in 100L water) for 5 days. The dried fillets (24 hr at 22°C) were further smoked for 24 hr under beech smoke at 22°C (85% relative humidity) in the smoking cabinet.

Color analyses in raw salmon extracts and in pure carotenoid solutions

Color analyses of acetone extracts of raw salmon flesh tissue and pure astaxanthin and canthaxanthin solutions were made with a Shimadzu UV-300 spectrophotometer (Sesakusho Ltd, Kyoto, Japan). Measurements were taken in 1 cm glass cuvettes. Pure carotenoid solutions were prepared from astaxanthin powder (5%) and dry canthaxanthin water-soluble powder (10%) (F. Hoffmann-LaRoche & Co. Ltd., Basle, Switzerland). The carotenoid (50 mg/L) was suspended in water (40°C) and further diluted by acetone/water mixtures. The spectrophotometer was connected to a computer (NORD 10/S, Norsk Data A/S, Oslo, Norway), able to register, store and calculate spectral data (Slinde *et al.*, 1982). Using the weighted-ordinate method (10 nm intervals), CIE (1976) L*a*b* values were calculated from the absorbance spectra in the range 380-760 nm (Hunter, 1975). With salmon color, L* corresponds to lightness, a* to redness, and b* to yellowness. The angle, H°_{ab} having tangent b*/a*, was calculated and used as a measure of the hue of the salmon color (Hunter, 1975; Hunt, 1977). Light source C was used for calculation.

Color analyses in raw, baked and smoked salmon flesh

Color parameters CIE (1976) L*a*b* and H°_{ab} in raw, baked and smoked salmon flesh were calculated from the reflectance spectra recorded by a HunterLab Labscan II sphere spectrocolorimeter (Hunter Ass. Lab. Inc. Virginia) in the region 400–700 nm, specular reflection included. A viewing area of 6.4 mm diameter was used. The illuminated area was 10 mm diameter. Samples of raw and smoked salmon were presented to the instrument as tissue plugs within white polyethylene caps (2.5 cm diameter, 1 cm height). The caps were covered on the sides and the back with aluminium foil. Baked salmon were minced with a fork before being put into the polyethylene caps. Samples were covered with a glass plate and measured four times, each sample being rotated 90 degrees between the measurements (Schmidt and Cuthbert, 1969; Little *et al.*, 1979). Color differences between pairs of salmon samples were calculated as $\Delta E^{*}_{ab} = [(L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ (Hunter, 1975).

Sensory analyses

A trained panel of 12 members performed visual sensory evaluation on eight pairs of salmon established according to carotenoid type and concentration, using a triangular test (Amerine *et al.*, 1965). Each panelist evaluated two combinations of each pair. Prior to the analyses, the panelists were trained in evaluating darkness (in comparison with black), color intensity and hue of color chips according to the Natural Color System (NCS) (SSI, 1979). The panelists were asked to ascribe differences within each pair of salmon to darkness, color intensity or hue (yellowish). With the triangular test no quantitative evaluation of color differences could be made. The sensory evaluations were conducted on both raw, baked and smoked salmon pairs. Samples were presented as 1 cm high tissue plugs, 2.5 cm in diameter, upon a white plate. Samples were viewed under white light (Colorette, 40W, Luma, Sweden).

Statistical analyses

Results from the quantitative analyses of salmon tail homogenates and results from the different color analyses were subjected to one-way analysis of variance or linear regressions. Differences in the slopes of the regression lines were tested according to the method of Draper and Smith (1981). The sensory analyses were evaluated as described by Amerine *et al.* (1965).

RESULTS

IN FARMED SALMON, 93% of the carotenoid content was identified as canthaxanthin and 7% as astaxanthin. In wild salmon, the main carotenoid was astaxanthin (above 90% in sea salmon, above 85% in the river salmon). The remaining unidentified fractions of the wild salmon contained carotenoids more polar than astaxanthin and canthaxanthin. The astaxanthin concentration of the 24 wild salmon averaged 11.3 mg/kg (s = 2.3) while the canthaxanthin concentration of the 15 farmed salmon averaged 6.5 mg/kg (s = 1.2). Thus, the carotenoid concentration of the wild salmon was higher and covered a wider concentration range than the farmed salmon.

Among the 16 individual salmon selected for processing and sensory and chemical analyses, no significant differences in dry matter, ash, protein or fat content of tail samples between farmed and wild salmon were found. The chemical composition of the wild and farmed salmon averaged 26.0% dry matter (s = 1.0), 1.3% ash (s = 0.1), 21.5% protein (s = 0.8) and 3.2% fat (s = 0.7). Carotenoid concentration of the 8 wild salmon averaged 10.6 mg/kg (s = 3.3) while that of the 8 farmed salmon averaged 6.4 mg/kg (s = 1.6).

Color parameters in pure carotenoid solutions and in raw salmon extracts

In pure astaxanthin and canthaxanthin solutions, lightness (L*) (Fig. 1A) decreased, while redness (a*) (Fig. 1B) and yellowness (b*) (Fig. 1C) increased with increasing carotenoid concentration of the solutions. Hue values (H°_{ab}) (Fig. 1D) decreased, giving more reddish solutions with increasing carotenoid concentration. Further, canthaxanthin solutions were lighter, less red, more yellow and had a more yellow hue than astaxanthin solutions of equal concentrations. With the conditions described, standard deviations of color parameters in carotenoid solutions were 0.1, 0.2, 0.4 and 0.8 for L*, a*, b* and H°_{ab}, respectively.

Color parameters of acetone/water solutions also depended upon solvent composition. By increasing acetone content from 75% to 99% in water, H°_{ab} values increased from 80 to 88 for canthaxanthin and from 78 to 83 for astaxanthin. The solutions thus became more yellow in hue with increasing acetone content of the solvent. The extraction conditions used for salmon flesh in the current experiment, resulted in acetone/water extracts with 90% acetone. Absorbance maxima in 90% acetone in water were 476 nm for astaxanthin and 474 nm for canthaxanthin.

The color parameters of the wild and farmed salmon extracts resembled those of their respective carotenoid solutions (Fig. 1). With lightness (Fig. 1A) and yellowness (Fig. 1C) no differentiation between astaxanthin and canthaxanthin containing extracts could be made. Both color parameters were significantly correlated ($P \leq 0.001$) to carotenoid concentration of the extracts, the correlation coefficient (r) for lightness on carotenoid concentration was −0.99, while that of yellowness (b*) was 0.99. Redness (Fig. 1B) and hue (Fig. 1D) of the salmon extracts, however, were clearly dependant upon type of carotenoid. As in the pure carotenoid solution, extracts containing astaxanthin had higher redness intensity and a more red hue than extracts containing canthaxanthin. Significant correlation between color parameters and pigment concentration ($r = 0.93$, $P \leq 0.001$) was found for redness in astaxanthin containing extracts only.

Color parameters in raw salmon flesh

Average color parameters L*a*b* and H°_{ab} values calculated from the reflectance spectra of raw flesh of the 16 individuals subjected for processing are presented in Table 1. Average standard deviations for color parameters of each salmon flesh sample when measured as described, were 0.2, 0.1, 0.2 and 0.5 for L*, a*, b* and H°_{ab}, respectively. As with acetone extracts (Fig. 1), increased pigment concentration caused significantly decreased lightness, increased redness and gave a more reddish hue to the flesh (Table 2). The most clear relationship was that demonstrated by the redness. With measurement made directly on raw flesh, no significant differentiation could be made between color parameters of wild and farmed salmon. Further, no significant correlations between caroten-

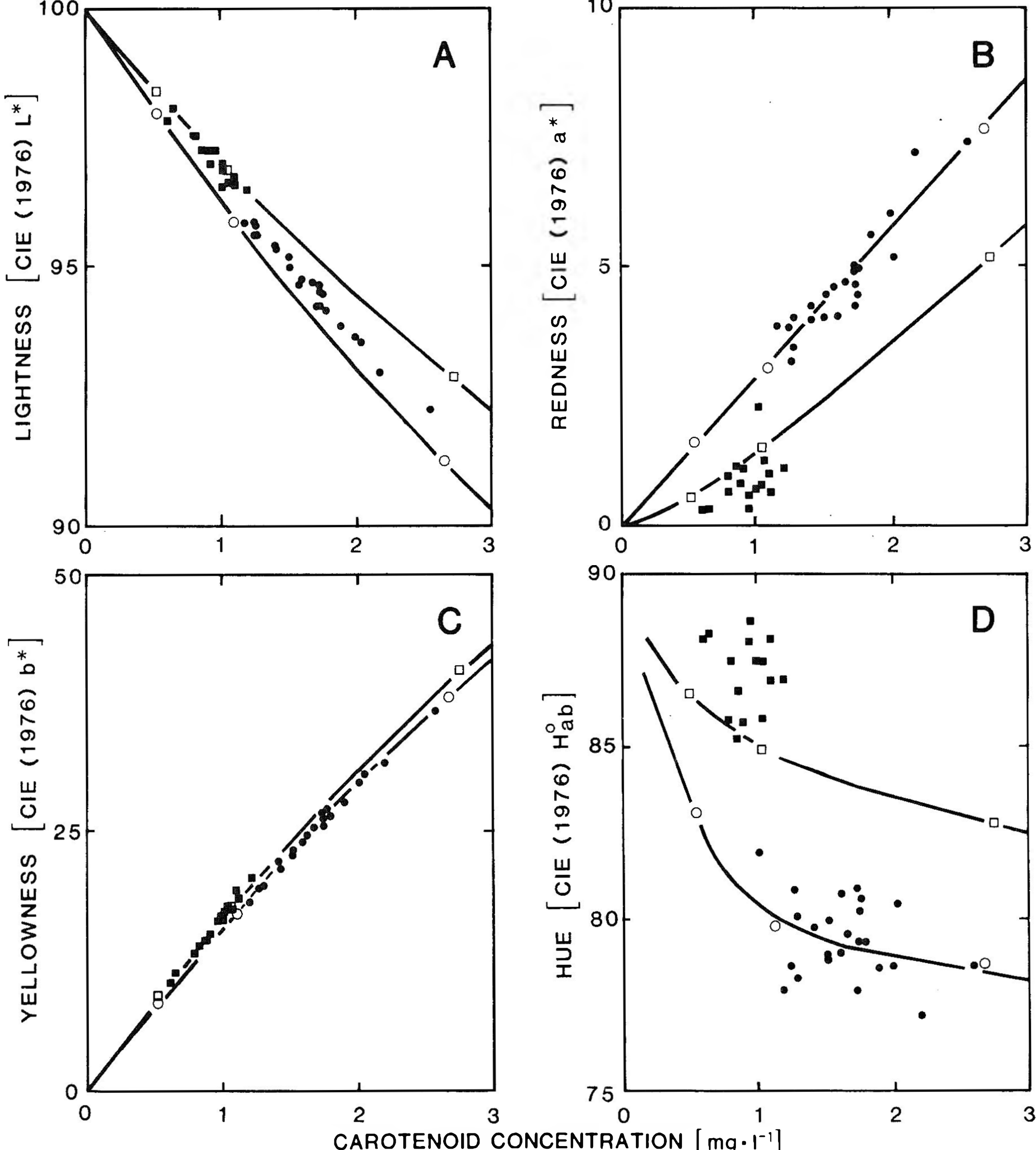

Fig. 1—Color parameters in pure carotenoid solutions and in acetone extracts from flesh of 39 salmon. Lines represent pure astaxanthin (○) and canthaxanthin (□) solutions, points represent wild (●) and farmed (■) salmon individuals.

oid content or color parameters and dry matter, fat or protein content of the raw flesh were found.

Color parameters in baked and smoked salmon flesh

The color of baked salmon flesh contained less redness and had a more yellowish hue than raw flesh (Table 1). The decrease in redness was more extensive in the farmed salmon (41%) than in the wild salmon (23%). As in raw fish, redness and hue in baked salmon were better correlated with carotenoid concentration of raw salmon than were lightness and yellowness (Table 2). Redness increased with increasing pigment concentration, causing a more reddish hue to the baked salmon flesh at higher concentrations. Significant correlations were found between redness and hue of baked ($r=0.75$, $P\leq0.001$) and redness and hue of raw ($r=0.70$, $P\leq0.01$) salmon flesh.

Smoked salmon flesh was lighter, had less redness and yellowness and was more reddish in hue than raw salmon (Table 1). The redness in particular, was significantly correlated to the pigment concentration of the raw salmon (Table 2). Lightness, redness and hue of smoked salmon were significantly correlated with the corresponding parameters of raw fish, the correlation coefficients being 0.66 ($P\leq0.01$), 0.63 ($P\leq0.01$) and 0.56 ($P\leq0.05$), respectively.

Sensory evaluation of raw, baked and smoked salmon

In the visual sensory analysis, wild and farmed salmon with differences in pigment concentration ranging from 0 to 6.4 mg/kg, were all judged as significantly different both as raw, baked and smoked samples. (Table 3). The panelists described the farmed fish as less dark, less colored and more yellowish in hue than the wild fish.

Comparison of raw and processed wild salmon with different

Table 1—Color parameters in raw, baked and smoked salmon flesh[a]

	Raw salmon	Baked salmon	Smoked salmon
Wild salmon			
Lightness, L*	42.9 ± 2.6	71.4 ± 1.9	46.1 ± 2.4
Redness, a*	10.1 ± 2.0	7.8 ± 2.7	7.3 ± 0.8
Yellowness, b*	10.0 ± 2.2	17.8 ± 0.9	6.2 ± 1.2
Hue, H°_{ab}	44.5 ± 3.0	66.7 ± 6.1	37.9 ± 4.8
Farmed salmon			
Lightness, L*	43.0 ± 1.6	73.3 ± 1.4	48.1 ± 2.1
Redness, a*	8.3 ± 1.0	4.9 ± 1.1	6.4 ± 1.4
Yellowness, b*	10.1 ± 1.2	17.7 ± 0.7	6.4 ± 1.8
Hue, H°_{ab}	50.6 ± 2.2	74.7 ± 2.9	44.6 ± 5.5

[a] Color parameters were calculated from reflectance spectra of the salmon flesh. Values given are mean ± standard deviation of 8 salmon, each with 4 replicate measurements per individual.

Table 2—Correlation coefficients (r) and linear regressions of color parameters of flesh of 8 wild and 8 farmed salmon on carotenoid concentration (C mg/kg) of raw salmon

	r	Regressions
Raw salmon		
Lightness, L*	−0.52*[a]	L* = 45.6 ± 0.3C
Redness, a*	0.91***	a* = 5.0 + 0.5 C
Yellowness, b*	0.41 n.s.	b* = 8.3 + 0.2 C
Hue, H°_{ab}	−0.69**	H°_{ab} = 54.8 − 0.9 C
Baked salmon		
Lightness, L*	−0.61*	L* = 75.3 −0.3 C
Redness, a*	0.87***	a* = 0.8 + 0.7 C
Yellowness, b*	0.54*	b* = 16.6 + 0.1 C
Hue, H°_{ab}	−0.85***	H°_{ab} = 84.2 − 1.6 C
Smoked salmon		
Lightness, L*	−0.56*	L* = 50.2 − 0.4 C
Redness, a*	0.74**	a* = 3.3 + 0.5 C
Yellowness, b*	0.34 n.s.	b* = 5.0 + 0.2 C
Hue, H°_{ab}	−0.49 n.s.	H°_{ab} = 48.8 − 0.9 C

[a] Asterisks give significance level: ***P≤0.001, **P≤0.01, *P≤0.05, n.s. P>0.05

Table 3—Visual sensory evaluation of wild and farmed salmon flesh[a]

Pair compared	Δcarotenoid mg/kg	Raw salmon		Baked salmon		Smoked salmon	
		ΔE^*_{ab}[b]	No.[c]	ΔE^*_{ab}	No.	ΔE^*_{ab}	No.
Wild/farmed	0.0	3.6	23***	3.6	23***	4.3	23***
(astaxanthin/	0.2	7.1	23***	3.0	18***	3.2	17***
canthaxanthin)	0.5	3.7	21***	3.3	22***	5.7	24***
	6.4	2.8	24***	5.5	24***	1.8	24***
Wild/wild	4.7	6.5	23***	3.8	23***	3.6	14**
(astaxanthin/ (astaxanthin)	7.7	6.1	24***	8.1	24***	7.4	24***
Farmed/farmed	0.3	3.3	15**	2.3	24***	4.8	18***
(canthaxanthin/ canthaxanthin)	2.0	2.8	15**	1.7	22***	2.1	7 n.s.

[a] Triangular test between pairs of salmon with specified differences (Δ carotenoid) in carotenoid concentration.
[b] Color difference (ΔE^*_{ab}) in each pair based on CIE(1976) L*a*b* values from reflectance spectra of salmon flesh.
[c] Number of correct answers in the triangular test (total = 24). Asterisks give significance level: ***P ≤ 0.001; **P ≤ 0.01; n.s. P > 0.05).

astaxanthin or canthaxanthin concentration revealed significant visual differences for all pairs except one smoked (Table 3). With both types of pigmentation, more darkness, higher color intensity and less yellowish hue were ascribed to samples having higher pigment concentrations.

DISCUSSION

THE WILD and farmed salmon had different genetic background, feeding, environment and slaughtering conditions. These factors may have affected the pigmentation, and differences in color should not be ascribed to differences in type and concentration of carotenoids alone. Further, since the experiments dealt with a limited number of fish, the results may not reflect all variation within wild and farmed salmon. Previous reports on the effect of cold (Schmidt and Idler, 1958) and frozen (Schmidt and Cuthbert, 1969) storage, indicate that less than 5 wk frozen storage, as in the present experiment, is not likely to have any influence on the color parameters of the salmon flesh. In accordance with the finding of Saito (1969), the carotenoid concentration of the tail part of each individual was regarded as representative of the pigment concentration of the various sections of the salmon used in the analyses.

When estimating carotenoid concentrations from absorbance measurements, the levels obtained depend upon the extinction coefficient, $E^{1\%}_{1cm}$, chosen for calculations. $E^{1\%}_{1cm}$ for all *trans*-astaxanthin in acetone, *n*-hexane and cyclohexane is slightly above 2100 (DeRitter and Purcell, 1981; Manz, 1983; Chen and Meyers, 1984). The corresponding value of canthaxanthin in *n*-hexane and cyclohexane is $E^{1\%}_{1cm} = 2200$ (DeRitter and Purcell, 1981). During the extraction of carotenoids from water-dispersible beadlets, some *cis*-isomerization takes place, reducing $E^{1\%}_{1cm}$ to an average of 1970 for canthaxanthin in cyclohexane (Bauernfeind, 1981). *Cis*-isomerization is also found in extracts from fish feeds (Foss *et al.*, 1984) and Atlantic salmon (Schiedt *et al.*, 1981). Since no exact $E^{1\%}_{1cm}$ value has been reported for the conditions of the present study, an average $E^{1\%}_{1cm} = 1900$ was used for both astaxanthin and canthaxanthin (Foss *et al.*, 1984) .

Due to several factors like nutrition, fish size, sexual maturation, and environmental factors, pigmentation of salmonids vary (Foss *et al.*, 1984). Carotenoid concentrations are, therefore, likely to vary both in wild and farmed salmon. The carotenoid concentrations obtained in the wild salmon of the present study were of the same magnitude as those reported by Schiedt *et al.* (1981), but higher than concentrations obtained by Saito (1969). The carotenoid concentration of the farmed salmon coincided with the results of Torrissen and Torrissen (1984) and Torrissen *et al.* (1984), but were higher than those reported by Storebakken *et al.* (1985).

Schmidt and Idler (1958) reported canned salmon with a higher oil content to have a more desirable color. In the present study no significant relationship was found between lipid content and carotenoid concentration of the flesh. This is in accordance with previous findings of Saito (1969) and Spinelli and Mahnken (1978).

The presentation of samples to the instruments during reflectance measurement has been reported to have conclusive effect on the color parameters obtained for raw and cooked salmon flesh (Little and MacKinney, 1969; Schmidt and Cuthbert, 1969; Choubert, 1982). This effect was demonstrated in the present study by an unpresented experimental series where color parameters of raw homogenized samples were measured. Compared with readings of uncut flesh, homogenized samples were lighter and had higher yellowness while redness remained unchanged.

The effect of sample presentation on the results is likely to be related to the translucency of the salmon flesh samples. As stated by Little (1964), translucency will cause various amounts of light to be trapped within the samples unless special precautions, like increasing the exposed area relative to the illuminated area, are taken. In the present study, the measuring conditions available apparently did not compensate completely for this trapping effect. When salmon samples and paper color chips (SSI, 1979) were matched visually, instrumental readings caused lower L* values, *i.e.* more darkness, with the salmon samples than with the paper chips. As long as such fundamental factors concerning sample presentation are not controlled, no conclusive and absolute levels of color parameters in salmon flesh can be obtained. Detailed procedures for sample preparation and measuring conditions should therefore be given when reporting color analyses on salmon flesh.

Coinciding with previous findings (Schmidt and Idler, 1958), instrumental measurements showed less intensity in redness and a more yellowish hue when salmon flesh was baked compared with raw salmon. The red color seemed to be better

retained during baking in the wild salmon of the present experiment.

The correlation found between redness and carotenoid concentration of raw salmon flesh, concurred with results reported by Schmidt and Idler (1958) and by Saito (1969). In experiments with canthaxanthin pigmented rainbow trout, Choubert (1982) also found a more reddish hue at higher pigment concentrations. In the present study increased redness with increasing pigment concentration was found in both raw, baked and smoked salmon within the concentration range of 4.3–16.7 mg carotenoid/kg.

Huang *et al.* (1970) reported a lower limit of 0.2 for the color difference ΔE for visual differentiation between samples of squash purees with added canthaxanthin. Included in the ΔE values are differences in the three color dimensions L, a and b. In the present study with salmon flesh, this limit for ΔE was exceeded for each pair of salmon presented to the sensory panel even when no difference in carotenoid concentration was detectable. In accordance with this, the panelist significantly detected visual differences within all pairs of farmed and wild salmon.

Except for one pair of smoked salmon, raw, baked and smoked canthaxanthin-pigmented farmed salmon were visually judged as more yellowish in hue than the wild salmon containing astaxanthin. This confirms our finding of a more yellowish hue in the acetone extracts of the farmed salmon and are in agreement with results reported by Saito and Regier (1971) and Spinelli *et al.* (1974) in raw brook trout (*Salvelinus fontinalis*) and rainbow trout, respectively. The results are in contrast to those obtained by Schmidt and Baker (1969) and Foss *et al.* (1984) in rainbow trout. In the present study, significant differences in hue between wild and farmed salmon were not found in the instrumental color analyses directly on salmon tissue. Since no effort was made to quantify the visual differences within pairs, no conclusions about the relationships between color parameters and visual color, as made by Little *et al.* (1979), can be drawn.

In conclusion the results of the present study demonstrated that canthaxanthin-pigmented farmed salmon was more yellowish in hue than astaxanthin-pigmented wild salmon. The two types of salmon could be differentiated either by visual sensory analysis or by instrumental color analyses of acetone extracts of the salmon flesh. In raw salmon, red color intensity of flesh could be predicted from carotenoid concentration of the raw salmon, while in baked and smoked salmon flesh, red color intensity was predictable both from carotenoid concentration and red color intensity of raw salmon. Thus, under practical industrial conditions, it should be possible to achieve farmed salmon products of desired color quality, if pigment deposition in the salmon during rearing and the conditions for processing are controlled.

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Comparison of the Sensory Quality and Oxidative Rancidity Status of Frozen-Cooked Mussels (*Mytilus edulis L.*)

RICHARD F. ABLETT and STEPHEN P. GOULD

ABSTRACT

Sensory acceptability and tissue-specific occurrence of oxidative rancidity was investigated among a commercial sample of frozen-cooked mussels and Prince Edward Island (P.E.I.) mussel meats held under a range of frozen storage regimes. P.E.I. mussels stored at −30°C or −60°C yielded the most acceptable sensory quality. Sensory and oxidative rancidity status of the commercial product most closely reflected P.E.I. samples held under marginal storage temperatures (−12°C). Digestive gland tissue demonstrated pronounced morphometric variability and yielded the highest oxidative rancidity values among both commercial and experimental samples. It was concluded that, in addition to marginal storage conditions, tissue-specific morphometric variability, probably resulting from differences in cultivation regions, might influence progression of oxidative rancidity and, hence, sensory quality among frozen-cooked cultivated mussels.

INTRODUCTION

THE LONG TERM viability and expansion of the cultivated mussel (*Mytilus edulis*) industry in North America, particularly in the regions of New England and Atlantic Canada will likely include the emergence of a range of processed secondary products. Among these, considerable interest has been expressed in the possibilities of frozen-cooked mussels which have been available in European markets for several years. A significant potential for these products may exist in North America because a major advantage of a frozen-cooked product over live mussels, lies in the convenience of handling and distribution at the consumer level. However, in developing processing technologies which support the cultivated mussel industry, there remains a need to overcome some specific problems of quality which may impact on the acceptability of mussel products appropriate for North American markets.

In a previous study, we described an incidence of sensory deterioration among frozen-cooked cultivated Prince Edward Island (P.E.I.) mussels stored under marginal frozen storage conditions (Ablett et al., 1986). This observation was accompanied by a progressive increase in the oxidative rancidity status of the product. The same study revealed an improved sensory and chemical stability for mussels stored at −30°C and this supported the likelihood that the organoleptic deterioration was associated at least in part, with lipid oxidative changes. Prior treatment with ascorbic acid and chelating agents proved effective in retarding the progression of oxidative rancidity in cooked mussels stored under marginal (−12°C) frozen storage temperature. Nevertheless, the antioxidant failed to prevent the decline of aroma and flavour characteristics over the experimental period. It is recognized that problems of this nature are commonly encountered among a wide range of frozen seafood products including shellfish and can be attributed to the presence in these marine commodities of a relatively abundant polyunsaturated lipid content which shows a pronounced tendency toward oxidative deterioration (Pastoriza et al., 1980).

In view of the implications of this problem in terms of consumer acceptance, it was considered important to further investigate and compare the oxidative rancidity status of cultivated frozen-cooked P.E.I. mussels with an existing commercially available product held under industrial cold storage conditions. Thus, the objective of the present study was: (1) to compare the sensory quality and oxidative rancidity status of cultivated 'Tusker Rock' brand frozen-cooked mussels with experimental samples of frozen-cooked P.E.I. mussels; (2) to compare morphometric parameters and determine oxidative rancidity values for specific tissue regions; (3) to establish and compare the composition of the major lipid classes between the intact mussel meats of commercial and experimental samples.

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MATERIALS & METHODS

Mussels

Live cultivated blue mussels were shipped directly to the Canadian Institute of Fisheries Technology, Halifax, Nova Scotia by the Atlantic Mussel Growers Association, Montague, Prince Edward Island in April, 1984. Following overnight storage at 4°C, mussels were steam-cooked (7-8 min) at atmospheric pressure in batches of 16 kg in a 100 liter steam-jacketed stainless steel kettle containing 5 liters of water. Immediately after steaming the meats, manually separated from shells, were cooled to 4°C and individually quick frozen on aluminum trays in a plate freezer at −35°C for a minimum period of 30 min. Following freezing, mussel meats were heat sealed in batches of 500g in polyethylene bags, stored at −12°C, −30°c, and −60°C, and examined 10 or 20 wk later.

Commercial samples of imported cultivated frozen-cooked mussels ('Tusker Rock' Brand, Lett and Co. Ltd., Wexford, Ireland) were obtained from a seafood broker in Boston (G.S. Higgins Ltd., Hanover, MA) where they had been held for a period not exceeding six months under commercial cold storage conditions (−18° to −20°C). Care was taken to ensure that the commercial samples were held frozen on dry-ice during transit to the Canadian Institute of Fisheries Technology, whereupon all samples were stored at −30°C prior to experimentation which was completed within 4 wk.

Sensory evaluation

In sensory analysis studies, both the commercial and P.E.I. samples were assessed on the basis of aroma, flavor and texture characteristics using a seven point hedonic scaling (Larmond, 1977) ranging from 'like very much' (7), 'neither like nor dislike' (4), to 'dislike very much' (1). All samples prepared for taste panel presentation were thawed at room temperature (23°C) and placed in groups of three in code-labelled glass petri dishes containing 2.5 mL distilled water. All petri dishes containing mussels were warmed in an oven at 220°C for 10 mins prior to sensory evaluation. In all instances, ten panelists, initially screened and stained for the purposes of mussel evaluation, were presented with samples under standardized fluorescent red light conditions. All panels were conducted in the sensory evaluation facilities of the Canadian Institute of Fisheries Technology.

Morphometric evaluation

Mussel meats randomly selected from the commercial and experimental samples were compared on the basis of total wet weight values. In addition, the digestive gland was separated from the body tissue of randomly selected individual meats, weighed and the digestive gland:body weight ratio calculated for all samples.

Table 1—Sensory evaluation comparison between Prince Edward Island and commercial frozen-cooked mussels[a]

	Prince Edward Island[b]			
	−12°C	−30°C	Commercial[c]	Reference[d]
Aroma	3.83[e]	4.07[e]	3.80[e]	4.16[e]
Flavour	2.92[e]	4.08[f]	2.70[e]	4.79[f]
Texture	3.98[e]	3.95[e]	3.50[e]	4.81[f]

[a] Sensory analyses conducted with 10 panelists. Hedonic scale values: (7) like very much; (4) neither like nor dislike; (1) dislike very much. Values represent the mean scores obtained from four panels.
[b] P.E.I. samples stored for 10 wk.
[c] 'Tusker Rock' brand, Lett and Co., Wexford, Ireland.
[d] Reference samples stored at −60°C for 10 wk.
[e,f] Values across rows with unlike superscripts are significantly different ($p<0.05$).

Table 2—Oxidative rancidity status of Prince Edward Island and commercial frozen-cooked mussels[a]

	Prince Edward Island		
	−12°C	−30°C	Commercial[b]
10 wk	11.1 ± 1.0[c,d]	8.9 ± 1.0[c]	13.86 ± 0.6[d]
20 wk	20.6 ± 0.6[c]	8.1 ± 1.1[d]	13.86 ± 0.6[e]

[a] TBA values represent the mean ± S.D. All assays were conducted in triplicate and values represent μmoles malonaldehyde/kg wet weight tissue.
[b] 'Tusker Rock' branch, Lett & Co., Wexford, Ireland. Storage period prior to analysis not known.
[c,d,e] Values across rows with unlike superscripts are significantly different ($p<0.05$).

Table 3—Oxidative rancidity status of tissue regions[a]

	Digestive gland	Gonad[c]	Intact mussel
Prince Edward Island	9.6 ± 0.5[d]	6.6 ± 2.5[e]	10.9 ± 0.3[d]
Commercial[b]	19.3 ± 0.5[d]	13.9 ± 1.3[e]	13.8 ± 0.6[e]

[a] TBA values represent the mean ± S.D. of triplicate assays. Values represent μmoles malonaldehyde/kg wet weight tissue.
[b] 'Tusker Rock' brand, Lett & Co., Wexford, Ireland.
[c] Gonad - refers to gonadal tissue and other remaining body tissues.
[d,e] Values across rows with unlike superscripts are significantly different ($p<0.05$).

Chemical analysis

Moisture content of mussel samples was determined by gravimetric difference following homogenization of frozen mussel meats for 1.5 min in a Cuisinart Food Processor (Cuisinart Inc., Greenwich, CT) and drying at 105°C for a period of 24 hours. Similarly, total lipid content was determined gravimetrically on homogenized mussel meats following lipid extraction according to the method of Bligh and Dyer (1959). Oxidative rancidity measured as 2-thiobarbituric acid (TBA) reactive substances was determined for intact mussel meats, excised digestive glands and the remaining body tissue fractions according to the method of Tarladgis et al. (1960). In addition, the composition of the major lipid classes was established for intact mussel meats following total lipid extraction as described above and chromatographic separation over a period of 45 min on S2-chromarods (Iatron Laboratories Inc., Tokyo, Japan) using a hexane: diethyl ether:acetic acid (97:3:1 v/v) solvent system. Lipid classes were quantified against standards by means of a semi-automated Iatroscan Model TH-10 system (Iatron Laboratories Inc., Tokyo, Japan) using flame ionization detection.

Statistics

The data obtained from sensory and chemical analyses was evaluated by analysis of variance and differences between means were determined by Tukeys test (Steel and Torrie, 1980). Morphometric data was evaluated by Students t-test. In all instances, mean values were considered different when $p<0.05$.

RESULTS & DISCUSSION

AS SHOWN IN TABLE 1, comparative evaluation of the sensory acceptability of frozen-cooked mussels revealed no differences in aroma between P.E.I. samples and the 'Tusker Rock' product ($p>0.05$). However, it was observed that the flavor characteristics of P.E.I. samples held at −12°C was comparable to that recorded for the commercial product ($p>0.05$). Both samples were less desirable than either P.E.I. mussels stored at −30°C or under reference conditions of −60°C ($p<0.05$). Essentially, no major differences of textural characteristics were apparent among the experimental samples ($p>0.05$) despite a raised moisture content ($p<0.05$) in the commercial product when compared to the P.E.I. samples (Table 4). Nevertheless, both the commercial and the P.E.I. samples scored lower ($p<0.05$) on the basis of texture characteristics than the reference samples stored at −60°C (Table 1).

Comparison of TBA values, as an indication of oxidative rancidity, revealed comparable results between experimental P.E.I. samples and the commercial product (Table 2). TBA values were highest ($p<0.05$) among the P.E.I. mussels stored at −12°C for 20 wk and these data were in agreement with the results of our previous study (Ablett et al., 1986). The oxidative rancidity status of the commercial samples was comparable to the values obtained for −12°C P.E.I. samples stored for 10 weeks and was higher ($p<0.05$) than TBA values recorded for P.E.I. mussels held at −30°C for either 10 or 20 wk. In recognizing that the observed oxidative rancidity status of the commercial sample is indicative of cold storage temperature under industrial conditions (−18° to −20°C), the elevated TBA values observed in the commercial sample and the P.E.I. mussels stored at −12°c probably explain the relatively poor sensory acceptability of the products on the basis of aroma and flavor characteristics (Table 1). Thus, the sensory acceptability and oxidative rancidity status of the commercial sample most closely resembled the performance of experimental mussels held at −12°C for 10 wk. The findings provide additional supportive evidence for the likelihood that oxidative rancidity does play an important role in the declining sensory quality and acceptability of frozen-cooked mussels (Ablett et al., 1986). The present results also indicate the problem may be of widespread occurrence since the commercial product was also prone to similar problems in this respect. As stated previously, no prior information pertaining to either temperature conditions or the duration of storage was available for the commercial sample. However, it is likely that the product had been stored for some months and under more marginal temperature conditions than the P.E.I. samples held at −30°C.

The digestive gland had the highest ($p<0.05$) oxidative rancidity status among the tissue components of both the experimental P.E.I. samples and the 'Tusker Rock' product (Table 3). This was of interest when compared with the lower values obtained for the remaining body tissue which is principally composed of gonadal tissue. This observation suggests that the digestive gland serves as a major source of off-flavor development among frozen-cooked mussel meats. Previous evidence has shown the digestive gland served as a specific storage organ for macronutrients, including triglyceride reserves, prior to subsequent distribution to other tissue regions (Bayne et al., 1976). Moreover, it is noted that the highly convoluted tubular structure of the digestive organ (Bayne et al., 1976) also exhibited an elevated membranous lipid profile, which could favor a greater progression of oxidative deterioration compared with other tissue regions. Extensive evidence has implicated oxidative rancidity among frozen seafood products to be closely associated with the degradation of polyunsaturated acyl chains derived from hydrolysis of membrane-derived phospholipids (Olcott, 1963; Shewfelt, 1981). Detailed comparative investigations are presently underway to determine whether lipid compositional profiles vary between specific tissue regions in mussels, and, if so, whether some organs would be more prone to oxidative deterioration than others. In this respect, a previous study has indicated that the macronutrient component of ingested plankton foodstuff also appeared capable of influencing the compositional profile of mussel meats (Drzycimski, 1961).

Morphometric comparison between the experimental P.E.I. mussel meats and those of the commercial product revealed no differences ($p>0.05$) in total wet weight, although it was noted that the moisture content of the latter was greater ($p<0.05$) than that of the P.E.I. samples (Table 4). Interestingly, the

Table 4—Morphometric comparison of Prince Edward Island and commercial frozen-cooked mussels[a]

	Wet Weight (g)			Moisture (%)
	Intact mussel	Digestive gland	Gonad	Intact mussel
Prince Edward Island	5.83 ± 2.1[c]	1.80 ± 0.3[c]	4.62 ± 1.8[c]	72.63 ± .004[c]
Commercial[b]	4.72 ± 1.4[c]	0.63 ± 0.1[d]	4.06 ± 1.2[c]	76.06 ± .07[c]

[a] All values represent the mean ± S.D. of 25 samples.
[b] 'Tusker Rock' brand, Lett & Co., Wexford, Ireland.
[c,d] Values in coumns with unlike superscripts are significantly different (p<0.05).

Table 5—Comparison of major lipid components[a]

Lipid class	Prince Edward Island	Commercial[b]
Triglyceride	60.2 ± 6.8	35.0 ± 2.8
Total phospholipids	35.7 ± 6.9	51.6 ± 3.8
Cholesterol	4.4 ± 0.4	12.8 ± 0.4

[a] Values represent the mean ± S.D. of four replicates expressed as percentage composition.
[b] 'Tusker Rock' brand, Lett & Co., Wexford, Ireland

digestive gland of P.E.I. cultivated mussels amounted to 20.8 ± 3.6% of total body weight; this was almost twice the size ($p<0.05$) of that observed in the commercial sample which accounted for 13.7 ± 2.1% of the total body tissues. The observation may reflect genetic differences or some developmental response to the nutritional or environmental profile of the respective cultivation regions. Thus, several previous studies have examined morphological differences among tubule types within the digestive gland of mussels and the evidence indicates the existence of considerable intra-specific variation for this organ (Robinson, 1981; Robinson et al., 1981). Moreover, the existence of pronounced genoadaptive differences among mussel stocks has also been established and it is likely that genetic variation imposes substantial morphological and developmental impacts on mussel populations cultivated in different regions (Gartner-Kepkay et al., 1983). Since oxidative rancidity was determined to be maximal in the digestive gland tissue of mussel meats, some problems of declining acceptability might be anticipated for frozen-cooked mussels derived from stocks which demonstrate pronounced development of this organ.

Some substantial variance in the profiles of the major lipid classes was observed between the two groups of intact mussel meats. Table 5, shows that the triglyceride fraction of the commercial product was almost half that determined for the experimental P.E.I. samples. In contrast, both the phospholipid and cholesterol levels of the commercial samples appeared greater than those of the P.E.I. samples. In evaluating the significance of these observations some caution is required, however, since our previous study demonstrated relatively wide variability to occur among the lipid content of cultivated mussels (Ablett et al., 1986). Other studies have shown the lipid profile of mussel meats varied on a seasonal basis (Slabyj et al., 1978) and the present observation may reflect differences in sexual maturation, nutritional status at the time of harvest or, possibly, degradative effects attributable to residual lipase activity during the period of frozen storage (Geromel and Montgomery, 1980). In the present study, no record of the harvest period of the commercial mussels was available. For this reason, only tentative comparisons can be made between the samples. Of interest, was the apparently large difference in total phospholipid values observed between the P.E.I. and commercial samples (Table 5). In view of the contribution of the phospholipid fraction in provision of polyunsaturated acyl chains in lipid oxidation pathways among frozen seafood, this fraction may eventually prove of major significance as an influence on the sensory quality and storage stability of frozen-cooked mussel meats. Further in-depth studies are presently underway to evaluate this possibility.

In summary, the results of the present study provide important comparative observations which support the likelihood that oxidative rancidity does indeed play a key role in the sensory decline of cooked-mussel meats held under frozen storage. The occurrence of relatively low sensory acceptability scores among marginally stored (−12°C) frozen P.E.I. mussels was also identified as a problem in a typical sample of retail product obtained from a commercial cold storage facility. The observation raises an important question as to the occurrence of deteriorative rancidity among other commercially available sources of frozen-cooked mussels and, hence, the overall perception of the product at the consumer level. Moreover, the finding that oxidative rancidity problems appear differentially disposed among specific tissue regions, notably the digestive gland, may prove of future significance among frozen mussel products derived from different cultivation regions. Thus, pronounced tissue specific morphometric differences between cultivated stocks, as determined in the present study, may exacerbate this problem. Future studies will examine in closer detail, the impact of specific tissue regions in the progressive development of oxidative rancidity for cooked mussels held under frozen storage. In addition, these studies will also attempt to establish the contribution of each tissue region to the overall sensory quality of the product.

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Effect of Environmental Salinity on Sensory Characteristics of Penaeid Shrimp

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ABSTRACT

White shrimp, *Penaeus vannamei*, acclimated to 10, 30 and 50 parts per thousand (ppt) salinities were evaluated for flavor by a trained sensory panel. The shrimp were also analyzed for tissue moisture, chloride, free amino acid concentration and free amino acid profile. Results of triangle tests showed a significant flavor difference between shrimp held at 10 and 50 ppt and 30 and 50 ppt but not between 10 and 30 ppt. On a wet weight basis, the free amino acid concentration was 81% higher in shrimp held at 50 ppt than at 10 ppt and 63% higher in shrimp held at 50 as compared to 30 ppt. However, between shrimp held at 30 as compared to 10 ppt the difference was only 11%. The difference in flavor intensity of shrimp held at different salinities appeared to be a direct reflection of the concentration of tissue free amino acids which serve as osmoregulators in shrimp tissue.

INTRODUCTION

THE FLAVOR of fish and shellfish is derived from extractable components which may be divided into two broad categories. The first category contains the nitrogenous compounds which include free amino acids, nucleotides and related compounds, low-molecular-weight peptides and quaternary ammonium bases. The second category is comprised of nonnitrogenous compounds such as organic acids, sugars and inorganic constituents. Of the nitrogenous compounds, free amino acids predominate in the majority of mollusks and crustaceans. Together with quaternary ammonium bases, free amino acids comprise nearly 90% of the total extractable nitrogen in molluskan and crustacean tissue (Konosu and Yamaguchi, 1982). Of nonnitrogenous compounds, Na^+, K^+, Cl^- and PO_4^{3-} are the major inorganic constitutents of crustacean tissue (Hayashi et al., 1981).

Free amino acids have been implicated as being responsible for the characteristic flavors of fish and shellfish (Simudu and Hujita, 1954; Hashimoto, 1965). In taste panel observations on Indian shrimp, *Metapenaeus dobsonii*, Rangaswamy et al. (1970) found that glycine contributed to the sweet flavor, whereas glutamic acid, leucine and proline conferred a general desirable flavor. Arginine was not found to affect flavor to an appreciable extent, while taurine added an unusual after-taste. Konosu et al. (1960) reported a flavor enhancing effect when free amino acids, other than histidine, in combination with inosinic acid were added to Katsuwobushi stock (dried bonito tuna preparation). Simudu and Hujita (1954) and Hashimoto (1965) determined that glycine is responsible for the sweet flavor of fresh shrimp, whereas histidine contributes a "meaty" characteristic (Simudu et al., 1953). In a series of taste test assessments of synthetic extracts simulating boiled snow crab (*Chionoectes opilio*) meat, Hayashi et al. (1981) determined that omission of either arginine, glutamic acid or glycine from the extract caused a distinct decline in sweetness and "mouth satisfying meaty taste." Arginine and glutamic acid were found to be especially indispensible for producing the taste characteristic of boiled crab. Alanine contributed to the sweetness but its contribution was far less than that of glycine.

In addition to free amino acids, inorganic ions such as Na^+ and Cl^- have also been found to contribute significantly to the flavor of marine products. Hayashi et al. (1981) determined that Na^+, K^+, Cl^- and PO_4^{3-} made indispensible contributions to the flavor of snow crab muscle extracts. Omission of Na^+ caused a dramatic decrease in sweetness and "mouth satisfying meaty taste" as well as a loss of crab-like taste. Omission of K^+ from the synthetic extracts produced a watery taste, although the crab-like taste was retained to some extent. Removal of Cl^- caused the extracts to become almost tasteless, while removal of PO_4^{3-} resulted in a decrease in sweetness, saltiness and "mouth satisfying meaty taste."

In penaeid shrimp, free amino acids (McCoid et al., 1984), Na^+ and Cl^- (Castille and Lawrence, 1981) have been shown to increase in the tail muscle with increasing salinity as a result of their role in osmoregulation. Since these same compounds impart particular flavor characteristics on marine food products, manipulation of the flavor intensity in penaeid shrimp may be possible through changes in environmental salinity. The purpose of this study was to determine the effect of environmental salinity on the sensory characteristics of penaeid shrimp.

MATERIALS & METHODS

Preparation and maintenance of aquaria

Circular 3800L fiberglass tanks were filled with sea water at 25–28 ppt salinity at 26–28°C. Four air tubes provided aeration to each tank. Since no bio- or undergravel filters were available, ammonia was carefully monitored. Dead shrimp and uneaten food were quickly removed to prevent deterioration of water quality. The shrimp were fed twice a day with a pelleted diet containing 38% protein. Feeding was not initiated until after 24 hr when the shrimp had been acclimated to the tanks.

Experimental design

White shrimp, *Penaeus vannamei*, were harvested from ponds at the Texas A&M University Shrimp Mariculture facility at Flour Bluff and transported in ice chests containing pond water (30 ppt, 23°C) to the Texas A&M Shrimp Mariculture Project Laboratory at Port Aransas, TX. Mean length and weight of the shrimp were 12.8 ± 0.08 cm and 16.11 ± 3.37g, respectively. The shrimp were randomly stocked into nine tanks at a density of 40–55 shrimp per tank. Following a 24 hr acclimation period, the salinity was changed to either 10, 30 or 50 ppt, with two to three tanks at each salinity. Salinity changes were performed at a rate of 4–8 ppt/day using appropriate amounts of rock salt or dechlorinated tap water. After the desired salinities had been reached, the shrimp were held in the tanks for 3 days, then harvested and deheaded. Since preliminary studies had indicated that shrimp frozen immediately postmortem had a metallic flavor, the shrimp tails used for these trials were held on ice for 24 hr then glazed, frozen and stored at −25°C until needed. Tail muscle samples of shrimp from each salinity treatment were analyzed for moisture, chloride, free amino acid content (FAA), amino acid profile and flavor characteristics.

Sample preparation

Frozen shrimp were thawed under cold running water and boiled in groups of ten in 800 mL of distilled water for 3 min. The cooked

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shrimp were then transferred to an ice bath for rapid cooking. Once cooled, the shrimp were peeled and held at 7–10°C until evaluated by the sensory panel.

Sensory evaluation

A ten-member discriptive attribute panel, selected and trained according to the procedures of Cross et al. (1978) and the American Society for Testing and Materials (ASTM, 1968) evaluated the shrimp for flavor characteristics and differences. The panel, selected from a pool of 24 volunteers (students and staff members), was composed of six females and four males, ranging in age from 24 to 34 years with a mean of 29.3 years. All members ate shrimp almost on a monthly basis and all but two panelists had previous taste panel experience.

Two types of analytical taste tests were performed: triangle tests (Larmond, 1977) and flavor analysis (quality rating or scalar scoring). Triangle tests were used to determine the presence or absence of flavor differences, as well as the degree of difference between shrimp from the three salinity treatments. Three comparisons were performed during the triangle tests: 10 vs 30 ppt; 10 vs 50 ppt; and 30 vs 50 ppt. Each panelist tested each comparison in triplicate.

Flavor analysis, utilizing an unstructured scale, was used to evaluate the flavor characteristics of shrimp from 10 and 50 ppt. The scale consisted of 6-inch (15 cm) horizontal lines with anchor points placed at 0.5 inches (1.3 cm) from each end. Panelists were asked to make a vertical line across the horizontal line to indicate the magnitude of the characteristic in question.

Testing was conducted over a three-day period, with two sessions per day (11:00 AM and 3:00 PM). The first five sessions consisted of triangle tests with two triangles presented at each session. Assignment of treatment pairs to be tested at a particular session was done at random as described by Sidel and Stone (1976). Flavor analyses were conducted during the final (sixth) testing session. This test was also completed in triplicate, with one pair tested in three trials. Samples were assigned to the panelists sequentially in the first two trials and randomized in the third.

Testing was performed in the Meat Science sensory testing facilities at Texas A&M University. Panelists were seated in individual booths that were connected to the food preparation area by a hooded dome through which the samples were passed. The booths were equipped with a napkin, pencil, appropriate questionnaires, unsalted crackers, rinse cup (distilled water at room temperature) and expectorant cup. The samples were coded with three-digit random numbers and served on white plastic foam plates. Serving temperature was 7–10°C (ASTM, 1973). Panelists were asked not to swallow the sample and to rinse with distilled water and eat an unsalted cracker between samples.

Chemical analysis

The shrimp were deheaded, peeled and deveined prior to analysis. The resulting tail muscles were analyzed for moisture, chloride (reported as sodium chloride), FAA (measured as amino acid nitrogen) and free amino acid profile. Moisture and chloride were determined according to AOAC (1980) procedures using air drying and volumetric methods, respectively. Amino acid nitrogen (AAN) was determined using a modification of the copper procedure of Spies and Chambers (1951) as described by Cobb et al. (1973). Shrimp extracts were prepared by blending shrimp in a "VirTis" 23 homogenizer at a ratio of 1g shrimp to 2 mL of 7% trichloroacetic acid solution. The mixture was then centrifuged and the supernatant removed and analyzed for AAN. Amino acid profiles were determined using an automated Beckman Model 120 C Amino Acid Analyzer. Shrimp extracts were prepared as for AAN determination then diluted 1:25 with distilled water and stored at −25°C until analyzed.

Statistical analysis

Data from the triangle tests were analyzed according to the procedures of Roessler et al. (1978). Degree of difference between treatments was determined by assigning numerical values to the ratings. Means were then calculated from these values. Data from flavor analyses were evaluated by assigning numerical values to each rating by measuring the distance of a panelist's response from the left of the line in units of 0.1 inches (0.25 cm). Values for each flavor characteristic were tabulated and analysis of variance conducted to detect significant flavor differences between samples.

Table 1—Triangle tests between shrimp grown in different salinities

Pairs tested	% Correct responses[a]	p-values[b]	Described degree of difference
10 ppt vs 50 ppt[c]	60	0.002	Moderate
10 ppt vs 30 ppt	43	0.116	Slight
30 ppt vs 50 ppt	60	0.002	Slight

[a] total of 30 judgements per pair tested
[b] Roessler et al. (1978)
[c] ppt = parts per thousand

Table 2—Moisture, chloride (as % NaCl) and free amino acid nitrogen (AAN) of shrimp used for taste tests

Salinity (ppt)[c]	Moisture (%)	NaCl (%) W[a]	NaCl (%) D[b]	AAN (mM/100g) W	AAN (mM/100g) D
10	77.88[d]	0.23[d]	1.02[d]	18.17[d]	82.16[d]
30	78.16[d]	0.38[e]	1.77[e]	20.17[d]	92.33[d]
50	75.57[d]	0.36[e]	1.48[e]	32.87[e]	134.54[e]

[a] Wet weight basis
[b] Dry weight basis
[c] ppt = parts per thousand
[d,e] Means within each column having the same letter are not significantly different (P>0.05).

Table 3—Amino acid profiles (%) of shrimp used for taste tests

Amino acid	Salinity 10 ppt[a] Molar %	30 ppt Molar %	50 ppt Molar %
Lysine	1.83	1.60	1.81
Histidine	ND[b]	0.26	0.35
Arginine	17.49	14.68	11.54
Taurine	1.86	1.79	1.29
Aspartic acid	ND	ND	0.32
Serine/Threonine	3.49	2.74	3.34
Glutamic acid	0.85	1.00	1.23
Proline	31.22	32.74	29.30
Glycine	38.57	38.83	34.77
Alanine	3.47	5.15	12.73
Valine	ND	ND	1.05
Methionine	ND	0.12	0.38
Isoleucine	0.39	0.37	0.64
Leucine	0.82	0.71	1.25
Tyrosine	ND	ND	ND
Phenylalanine	ND	ND	ND

[a] ppt = parts per thousand
[b] ND = Not detected

RESULTS & DISCUSSION

RESULTS of triangle tests between shrimp held at different salinities are shown in Table 1. According to statistical tables for estimating significance of triangle tests (Roessler et al., 1978), a minimum of 15 (nearest integer) correct responses out of 30 evaluations are required to establish significance at the 5% level. A significant difference in flavor was thus found between shrimp held at 10 and 50 ppt and between shrimp held at 30 and 50 ppt. The difference in flavor intensity of shrimp held at different salinities appeared to be a direct reflection of the tissue free amino acid concentration. This is exemplified by the compositional data shown in Table 2. The FAA concentration on a wet weight basis was 81% higher in shrimp held at 50 ppt as compared to shrimp held at 10 ppt and 63% higher in shrimp held at 50 as compared to 30 ppt. However, between shrimp held at 30 as compared to 10 ppt the difference in FAA was only 11% which the panel was not able to detect at a 5% level of significance.

Glycine, proline, arginine, serine/threonine and alanine were the predominant FAA at the three salinities tested. Together these amino acids comprised 94% of the FAA pool (Table 3). Serine and threonine are reported together since both eluted from the column at approximately the same time (103–106 min) and thus were difficult to quantify. However, since Simpson et al. (1959) were unable to detect threonine in *Penaeus aztecus*, the amino acid is most probably serine. The amino acid profiles generally compare favorably with those obtained

Table 4—Mean intensities of the various flavor characteristics

Flavor Characteristic	Salinities		p-values
	10 ppt[c]	50 ppt	
Shrimpiness	2.61[a]	3.25[b]	0.0017
Sweetness	1.81[a]	3.23[b]	0.0001
Chemical-metallic	0.95[a]	0.80[a]	0.4825
Nuttiness	1.28[a]	1.98[b]	0.0010
Saltiness	1.17[a]	1.50[b]	0.0169

[a,b] Means within each row having the same letter are not significantly different (P>0.05).
[c] ppt = parts per thousand

by McCoid et al. (1984) for *P. vannamei*. At the salinities they tested (10, 20, 30, 40, 50, and 60 ppt), the same amino acids comprised 93–96% of the total FAA pool. With exception of serine/threonine, this was also true for *Macrobrachium rosenbergii* acclimated to different salinities (Tan and Choong, 1981). Although the FAA concentration increased with increasing salinity, McCoid et al. (1984) found that the relative contribution of individual FAA to the total amino acid pool was not constant. Molar percentages of glycine and serine/threonine varied randomly with salinity, with the concentrations of arginine and proline inversely related to salinity. Similar results were observed in the current study.

Results of flavor analysis between shrimp grown at 10 vs 50 ppt are shown in Table 4. Shrimp held at 50 ppt were found to be significantly sweeter, shrimpier, nuttier and saltier than shrimp held at 10 ppt. There was no significant difference in the chemical-metallic flavor characteristic. Since glycine contributes to the sweet flavor of shrimp (Rangaswamy et al. 1970; Simudu and Hujita, 1954; Hashimoto, 1965), increases in the glycine concentration could account for the more intense sweet flavor the taste panel described for shrimp held at 50 ppt. In addition, alanine, which also confers a sweet flavor (Hayashi et al., 1981), showed the greatest relative increase on a molar basis when the environmental growth salinity was increased. The more intense shrimpiness as described by the panel for shrimp held at 50 ppt can be accounted for by the increase in glutamic acid at this high salinity. Increases in tissue salt content were also reflected by a significant increase in saltiness between shrimp held at 50 ppt as compared to 10 ppt.

CONCLUSIONS

FLAVOR EVALUATIONS of shrimp grown at different salinities revealed that manipulation of the environmental growth salinities affected the flavor of penaeid shrimp. Since free amino acids are major osmoeffectors in shrimp and also primary flavor producers in marine products, more flavorful shrimp can be produced by acclimation to high environmental salinities. Taste tests performed during this study statistically verified this observation.

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Evaluation of Thiol Activated Proteases from Clam Viscera as a Rennet Substitute for Cheese-Making

H. C. CHEN and R. R. ZALL

ABSTRACT

Clam rennet, which is a crude enzyme preparation of cathepsin B-like protease from clam viscera was characterized and compared to porcine pepsin and calf rennet for its suitability as a milk coagulant in cheese-making. Clam rennet was more proteolytic and produced a softer curd than the other two coagulants. However, influences of the pH and temperature on milk clotting with clam rennet were very similar to those of calf rennet. The cheddar cheese made from clam rennet was not inferior to the Cheddar cheese made from calf rennet. Quality enhancement occurred despite the view that high ratio of proteolytic to clotting activity is generally considered to be unfavorable for cheese-making. The higher proteolytic activity appeared to accelerate the ripening process. A small yield loss occurred as a result of excessive proteolysis during cheese-making.

INTRODUCTION

THE COAGULANT traditionally used for cheese-making in most of the world is the rennet extract from the abomasa of 10 to 30-day-old milk-fed calves. There has been a continued interest in finding calf rennet substitutes, because a world-wide shortage of calf rennet has arisen from an increase in cheese-making combined with a decline in the number of calves slaughtered (Green, 1977). For instance, cheese production in the U.S.A. increased from about 0.68 million tons in 1960 to 1.2 million tons in 1973, with a corresponding decrease in commercial calf slaughter from about 8 million to about 2 million. Even if complete utilization of calf stomach for rennet production had occurred, the potential supply would have been insufficient by 1969. It was suggested that in 1977 no more than 15% of cheese made in the U.S.A. was made with calf rennet alone. Of the cheese made in 1977, some 40% was made with calf rennet-pepsin mixtures and 40% from *Mucor* rennets. It is likely that the proportion made from calf rennet alone has decreased even further since then (Cheeseman, 1981). To meet demands, substitute products were developed and currently several calf rennet substitutes are commercially available. These are fungal rennets from *Endothia parasitica*, *Mucor miehei* and *Mucor pusilus*, 50:50 calf rennet and pepsin mixture, Bovine rennet and chicken pepsin. Commercial use of chicken pepsin as milk coagulant can only be found in Israel. In Canada, porcine pepsin is much more used than bovine pepsin or chicken pepsin. Porcine pepsin is advantageous because of availability, cost and instability after clotting.

Despite the existence of these substitutes, traditional calf coagulant is still preferred by most cheese manufacturers, because two particular problems arise in the use of rennet substitutes which have to be overcome for satisfactory cheese-making: (1) Losses of proteins are often greater during cheese-making with rennet substitutes, and this will lower the yield. (2) Differing proteolytic specificity may alter the ripening characteristics of the cheese, giving rise to off-flavors, and texture deterioration. Chen and Zall (1986) reported the isolation and characterization of cathepsin D-like and B-like proteases from the viscera of surf clam. In this study we investigated the possibility of using cathepsin B-like protease as a rennet substitute for cheese-making.

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MATERIALS & METHODS

Milk coagulant preparation

Calf rennet. Commercial single strength calf rennet (Pfizer Inc., New York, NY) diluted 1:20 with water was used for milk clotting time determination. The same stock rennet solution diluted 1:40 was used for cheese-making. All dilutions were made 5 min before use.

Porcine pepsin. A 0.2 mg/ml solution of twice crystallized and lyophilized powder of porcine pepsin (Sigma Co., St. Louis, MO) was used to measure milk clotting time. The solution was prepared daily in 0.005 M citrate-phosphate buffer of pH 4.2.

Clam rennet. A crude enzyme extract was prepared as described previously (Chen and Zall, 1986). This crude enzyme extract was further acidified and concentrated 10× according to the initial purification scheme also described previously (Chen and Zall, 1986), except that no cysteine-HCl was added and PM 30 membranes replaced XM 50 membranes in the ultrafiltration concentration step. Forty milliliters of the concentrate was then fractionated with ethanol. The precipitates from the 40–55% ethanol fraction were dissolved in 8 mL 10 mM cysteine-HCl solution. The pH of the cysteine-HCl solution was adjusted to 4.2 with 0.5N NaOH before use. This newly prepared solution was designated as clam rennet and allowed to stand at room temperature for 30 min before use.

Milk clotting time determination

The clotting activity of milk coagulants was determined by the method of Berridge (1952) with modifications. To 0.05 mL (unless otherwise mentioned) milk coagulant in a 25 mL test tube was added to mL substrate solution tempered to 30°C. The substrate solution was 10% reconstituted skim milk powder containing 10 mM $CaCl_2$, pH approximately 6.2. Where indicated, the pH of the substrate solution was adjusted with 0.5N NaOH or HCl while agitating. The mixture of coagulant and substrate was incubated in a 30°C water bath unless specifically indicated. Near the expected clotting time, which was determined by a preliminary test, the test tube was tilted ~60° at intervals of 5 sec. The moment of the first flake appearance in the liquid flowing down the tube was recorded as milk clotting time.

Proteolytic activity determination

Milk coagulants were assayed for proteolytic activity according to the methods described by Chen and Zall (1986).

Curd firmness determination

Clam rennet (0.5 mL), 1.25 mL porcine pepsin and 0.5 mL 1:20 diluted calf rennet were diluted to 2 mL with water and placed in 250 mL beakers. To each of these coagulants was added 100 mL tempered whole milk with pH adjusted to 6.5 with 0.5N HCl. The mixtures were then incubated at 30°C in a water bath. The amount of each coagulant indicated above would clot the milk in 12 min ± 10 sec. The firmness of the curd was determined at specific intervals after coagulation by an Instron Universal Testing Instrument, model TM (Instron Corporation, Canton, MA). A special curd knife meeting the specifications of Milk Industry Foundation (1959) curd tension determination method was attached to the Instron. The Instron, being calibrated to a range from 0–50 or 0–100 gram, was allowed to travel automatically at a speed of 50.8 cm/min to drive the knife into the curd three-quarters in depth of the coagulum, then pulled back at same speed. The recorder chart speed was 25.4 cm/min. Relative curd firmness was measured by the area graph weight of the xeroxed copy of the recorder chart.

Cheddar cheese manufacture

Two portions of pasteurized milk, each 13.636 kg, were made into Cheddar according to the method of Kosikowski (1977). Both clam rennet and calf rennet were used to make Cheddar cheese. The same procedures were followed, except that the renneting-to-cut time of clam rennet was prolonged to 50 min instead of 25 min, as commonly done with calf rennet. The amount of clam rennet used to make cheese had a clotting time equal to that of calf rennet at pH 6.5. However, prolonged renneting-to-cut time was required for clam rennet because of its slower curd firming rate. Small wheels of finished cheeses were waxed and allowed to ripen in a 10°C curing room.

Cheese yield and composition determination

Cheese yield was determined gravimetrically before waxing. The moisture contents of milk, cheese and whey were determined by the atmospheric oven method (Bianco et al., 1978b). Fat contents were determined by modified Babcock methods (Bianco et al., 1978a). Protein contents was calculated by multiplying the percent Kjeldahl nitrogen by 6.38. Samples were prepared according to Rowland's (1938a, 1938b) method for the determination of total Kjeldahl nitrogen and nonprotein Kjeldahl nitrogen.

Determination of soluble nitrogen, tyrosine and tryptophan in cheese extract

The amounts of soluble nitrogen, tyrosine and tryptophan in a clear sodium citrate-hydrochloric acid extract of cheese were determined according to the method of Vakaleris and Price (1959).

Alkaline urea-polyacrylamide gel electrophoresis of cheese samples

Samples were taken from both cheeses prepared with clam and calf rennets every 2 wk and kept frozen at −20°C. After 8 wk, 0.2g of cheese from each sample was mixed with 2 mL modified Poulick's buffer (Kiddy, 1975). The mixture was placed in a boiling water bath for 15 min to dissolve cheese proteins. Sample preparation and electrophoresis were then carried out according to the alkaline urea-polyacrylamide method as described by Kiddy (1975). Thoroughly destained gels were scanned in an E-C densitometer (E-C Corporation, St. Petersburg, FL) interfaced with Apple II plus computer (Apple Computer Inc., Cupertino, CA), which was loaded with LAB DATA MANAGER 1 (LDB1) software (Interactive Microware, Inc., State College, PA), to plot the relative peak heights of the protein bands.

Sensory evaluation of cheese

Ten-week-old cheeses were evaluated by preference tests. Ten panelists randomly selected from graduate students of the Department of Food Science were provided four different symbol-coded cheese samples. Each sample weighed about 20g. Two samples were from clam rennet cheese and the other two from calf rennet cheese. Samples were randomized among panelists. The panelists were divided into two groups and each group evenly seated around a 6' × 3' table in a clean and lighted room. Each panelist was asked to rate the acceptability of flavor, texture and body, and overall acceptability of the samples on a 9-point hedonic scale where 9 = like extremely and 1 = dislike extremely. Water was provided for each panelist to rinse the mouth after each taste.

RESULTS & DISCUSSION

Active clotting component of clam rennet

In the previous paper (Chen and Zall, 1986) cathepsin B-like and D-like proteases were discovered in the 40–70% ethanol fraction of clam viscera extract. It was also found (but not reported) that the 40–55% and 55–70% fractions were richer in B-like and D-like proteases respectively. The later fraction was relatively inactive in clotting milk in comparison to the former fraction. Thus, the 40–55% ethanol fraction was used exclusively in cheese-making studies. Clam rennet had to be activated in cysteine solution before use, and the activation curve is shown in Fig. 1. The increase in clotting activity during the incubation of enzyme preparation in cysteine solution was a result of thiol activation of cathepsin B-like protease rather than from pH activation of gastric or cathepsin D-like proteases. If proenzymes of gastric or cathepsin D-like proteases existed, they should have been activated by the low pH employed in the early stages of enzyme preparation. Furthermore, as shown in Fig. 1, the clotting activity of clam rennet was almost completely restored by cysteine solution after its activity was completely lost upon storage at 4°C for 72 hr. This evidence undoubtedly suggested that the major milk-clotting component in clam rennet was a thiol protease such as the cathepsin B-like protease rather than gastric or cathepsin D-like protease.

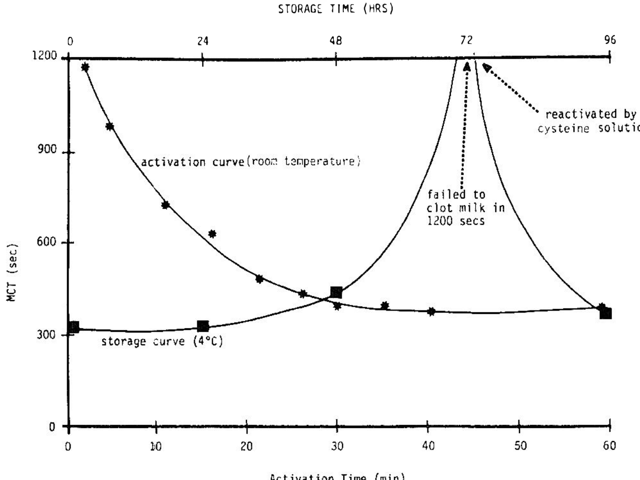

Fig. 1 — Activation and storage stability of clam rennet as indicated by milk clotting time (MCT).

Table 1—Relative ratio of clotting[a] to proteolytic[b] activity of different rennets at 30°C

Milk coagulant	Substrate: Hemoglobin	Casein
Calf rennet	1.00 (3.7[c])	1.00 (6.2[f])
Porcine pepsin	0.30 (1.8[d])	0.24 (6.2)
Clam rennet	0.13 (2.8[e])	0.03 (6.2)

[a] Clotting activity is defined as the reciprocal of milk clotting time under clotting assay conditions.
[b] Proteolytic activity is defined as the increase of OD 280/min under proteolytic assay conditions.
[c] Optimum pH of chymosin (Foltmann, 1970).
[d] Optimum pH of porcine pepsin (Ryle, 1970).
[e] Optimum pH of cathepsin B-like clam protease.
[f] pH of milk clotting assay.

Characterization of milk coagulants

Milk clotting to proteolytic activity ratio. The relative ratios of milk clotting activity to the proteolytic activity towards hemoglobin or casein are shown in Table 1 for all three coagulants. The ratio of clotting to proteolytic activity of calf rennet was arbitrarily taken as one unit for each substrate. The ratios of the other two coagulants were then standardized against these units. Clam rennet had a relatively lower ratio of clotting to hemolytic activity at optimum pH of each individual enzyme. The relative ratio of clotting to caseinolytic activity at milk clotting pH was examined, since this condition approximated more closely the actual process during cheese-making. Clam rennet had an even lower relative ratio of clotting to proteolytic activity under this condition. These observations indicate that clam rennet was more active in protein hydrolysis than equivalent clotting units of porcine pepsin or calf rennet. Calf rennet is considered as an ideal coagulant for cheese production because of its specific ratio of clotting activity to general proteolytic activity (Green, 1972). Martens and Naudts (1973) reported that most substitutes were more proteolytic than calf rennet relative to their clotting activity. Excessive proteolysis caused by these substitutes not only lowered the cheese yield and retention of fat by curd, but also had unde-

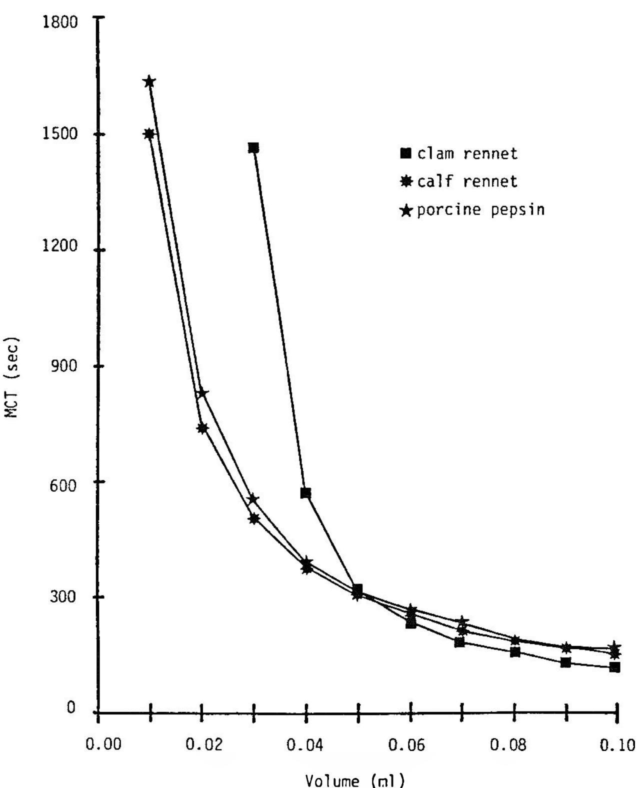

Fig. 2 — The quantity effect of coagulant on milk clotting time (MCT).

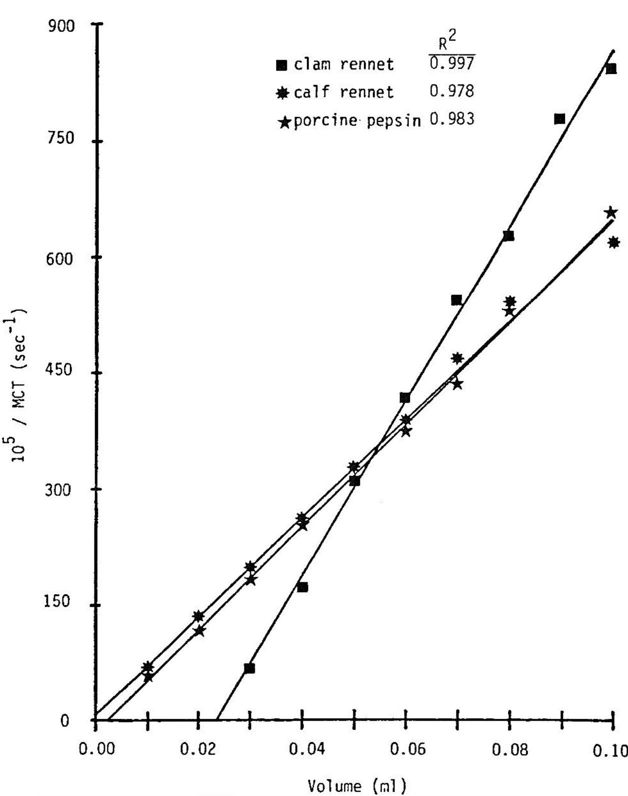

Fig. 3 — Linear regression of milk clotting rate (1/MCT) against coagulant quantity.

sirable effects on the body and flavor of the finished cheese. However, lack of proteolytic activity during ripening also resulted in textural defects and slow flavor development (Melachouris and Tuckey, 1964; Maragoudakis et al., 1961).

Coagulant concentration effect on milk coagulation. The influence of coagulant amount on milk clotting time for different coagulants is shown in Fig. 2. All three coagulant preparations had equivalent clotting activity (i.e. same clotting time) when 0.05 mL of each coagulant was used. However, the clotting time of clam rennet was significantly shorter than the other two coagulants when larger amounts of coagulant were used. On the other hand, the clotting time of clam rennet increased more rapidly as smaller amounts of coagulant were used and clam rennet failed to clot milk at the 0.02 mL level. This suggests that the reaction mechanism of clam rennet might differ from those of porcine pepsin and calf rennet, since clam rennet contains mainly a thiol protease which differs from carboxyl proteases, such as pepsin and chymosin, in catalytic mechanism. Another possibility is that natural inhibitors of clam rennet exist in milk. A heat-labile thiol protease inhibitor was isolated from raw milk (Reimerdes et al., 1976) after the discovery of more extensive proteolysis by plant proteases, such as papain, ficin and bromelain, in heat-treated milk than nonheat-treated milk (Klostermeyer et al., 1975). The event of concentration-dependent activity may be further confounded by the effect of pH on enzyme stability. Since clam rennet is relatively stable at pH 6–7 (Chen and Zall, 1986), in comparison to pepsin, the inability of clam rennet to coagulate milk at low enzyme concentration is probably not caused by the pH effect on the stability of clam rennet.

The data in Fig. 2 were replotted in Fig. 3 using milk clotting rate, which is the reciprocal of milk clotting time. The reaction rates of all three enzyme-catalyzed reactions were proportional to the amount of coagulant used, as indicated by the values of goodness of fit (R^2). However, the clam rennet intercepted the x-axis at a value significantly different from zero. When the presence of inhibitors is taken into account, the value of this intercept, i.e. about 0.0234 mL is the amount of clam rennet required to saturate the inhibitors. One more interesting observation was that the rate constant for clam rennet clotting was almost twice as high as those of porcine pepsin and calf rennet, as indicated by the slopes of Fig. 3. Calf rennet and porcine pepsin have similar rate constants, probably due to their similar catalytic mechanisms. Both calf rennet and porcine pepsin are carboxyl proteases and preferably attach the phe(105)-met(106) bond of kappa-casein (Dalgleish, 1982). Although many proteolytic enzymes will induce clotting of milk, this need not be taken as implying similarity in their mode of action to the carboxyl proteases. Since micellar instability is caused by the splitting of kappa-casein in the region of 105–106 peptide bond, it may be assumed that any enzyme capable of hydrolyzing kappa-casein at approximately this position should prove efficacious as a clotting agent (Dalgleish, 1982). Clam rennet is a thiol protease, which tends to have broader specificity and attacks several bonds at similar rates (Barrett, 1977). Thus, the relatively higher rate constant of clam rennet might result from its broader specificity.

pH effect on the clotting activity of milk coagulant. The clotting activity of all three coagulants appeared to be proportional to the pH (Fig. 4). Porcine pepsin had a lower pH optimum so that an increase in hydrogen ion concentration is more likely to promote the action of porcine pepsin than clam or calf rennet. The clotting activity of porcine pepsin decreased faster than the other two coagulants as pH increased. Clam and calf rennet had similar pH profiles of clotting activity. The absolute value of the x-intercept is the pH value that will cause complete inactivation of that coagulant before clot formation under the assay conditions. Porcine pepsin was unable to clot milk at pH 6.8 or above under the conditions studied, probably due to the complete inactivation of the enzyme before the clot could be formed, since porcine pepsin is unstable at pH > 6.0.

Temperature effect on the clotting activity of milk coagulant. The Arrhenius equation, $k = Ae^{-E/RT}$, relates the rate constant k to activation energy E and absolute temperature T. A is the frequency factor and R is the gas constant. Since the same amounts of coagulant were used for all temperatures,

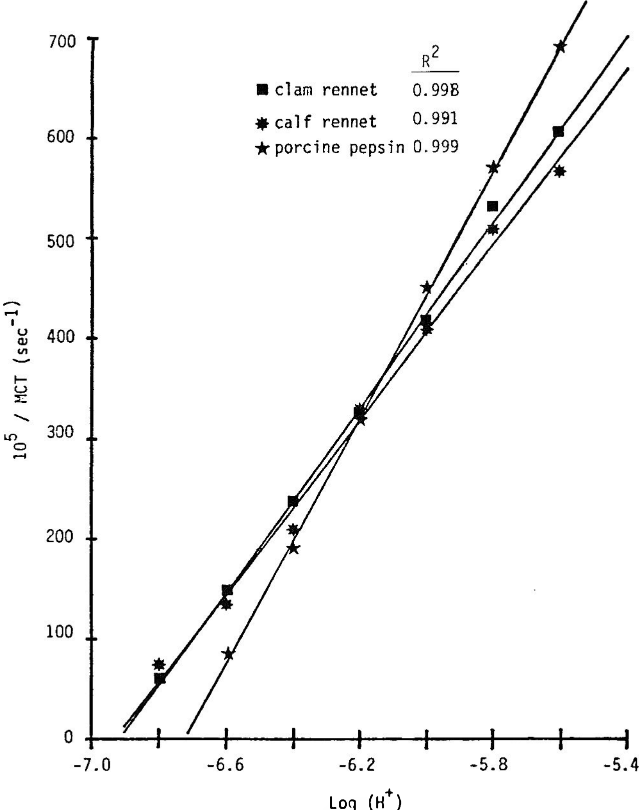

Fig. 4 — Linear regression of milk clotting rate (1/MCT) against the logarithmic concentration of hydrogen ions.

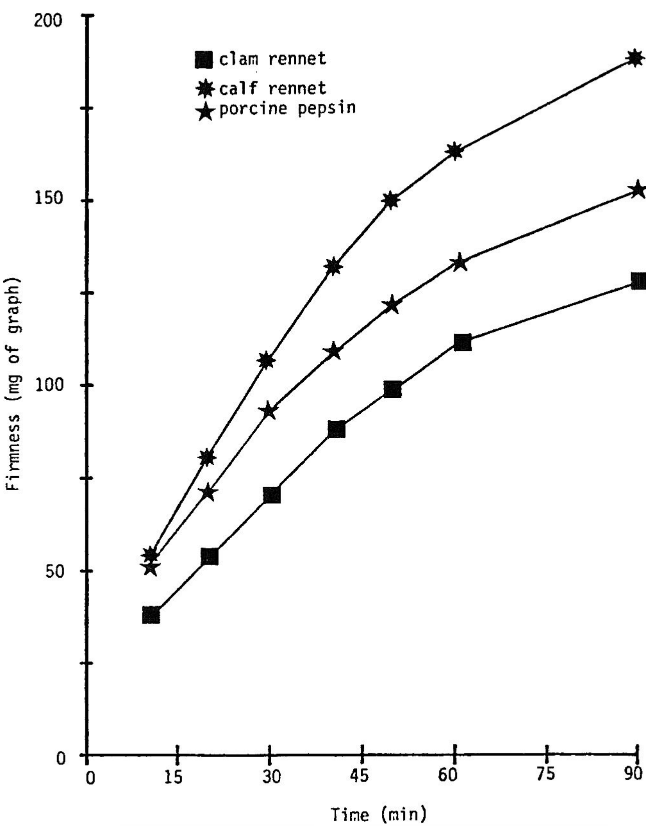

Fig. 6 — Increase of curd firmness (measured by graph weight) with time after coagulation by milk coagulant.

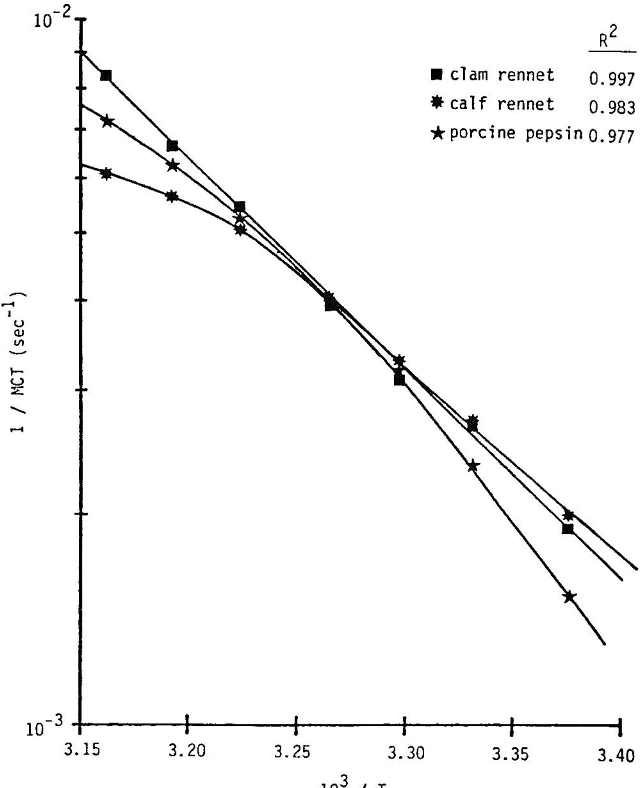

Fig. 5 — Arrhenius plot of milk clotting rate (1/MCT) vs. the reciprocal of absolute temperature (1/T).

the change of milk clotting rate with temperature reflects the change of rate constant with temperature. Fig. 5 is an Arrhenius plot of milk clotting rate and absolute temperature. All three coagulants had similar clotting activity in the temperature range from 30° to 37°C. Clam rennet more closely obeyed the Arrhenius rule over the entire temperature range examined as indicated by its higher goodness of fit. This observation suggests that clam rennet has a broader temperature optimum and/or stability since the catalytic reactions did not obey the Arrhenius relation above the temperature optimum.

Curd firmness. The firming of different curds with time are shown in Fig. 6. There was substantial difference in curd firmness among all three coagulants 90 min after coagulation. For satisfactory cheese-making, it is essential that the coagulant and conditions used will produce a curd of desired physical properties. The firmness and syneresis should be similar to those when calf rennet is used and there should be no significant loss of fat or protein at these stages. Porcine pepsin and clam rennet were inferior to calf rennet. Although there is evidence which suggests that the rate of curd firming is not important in determining the properties of the curd (Green, 1977), it is important to cut curd at the correct firmness so that the whey drains properly and the loss of milk solids is as low as possible (Green, 1977). Thus, it seems to be necessary to extend the clot-to-cut time when clam rennet or porcine pepsin is used as coagulant. This approach was used in the cheese-making trials as described in the Materials and Methods. However, the disadvantage of this approach is that more proteolysis will occur and the loss of non-fat milk solids is still unavoidable. Firmness of the clotted milk is affected by the type of milk coagulants along with many other factors, such as temperature, pH and calcium ion concentration. Addition of calcium chloride to increase curd firmness is a common practice of the cheese industry to overcome this problem.

Cheddar cheese from clam and calf rennet

Yield and composition of cheese. The percentage yield and composition of Cheddar cheeses made from clam and calf rennets are shown in Table 2. After the adjustment of moisture,

Table 2—Yield and composition of cheddar cheese

Coagulant	Yield (%)	Composition: Moisture (%)	Protein (%)	Fat (%)
Calf rennet	10.19	37.03	25.16	33.75
	(10.35)[a]	(38.00)	(24.77)	(33.23)
Clam rennet	10.19	38.08	24.13	33.75
	(10.18)	(38.00)	(24.16)	(33.79)

[a] The value in parentheses is adjusted to 38% moisture.

Table 3—Composition of rennet milk and whey

Coagulant	Moisture (%)	Fat (%)	N × 6.38: Total (%)	Nonprotein (%)
Renneted milk				
Calf rennet	87.94	3.60	3.28	0.268
Clam rennet	87.93	3.60	3.31	0.296
Whey				
Calf rennet	93.16	0.12	1.02	0.292
Clam rennet	93.14	0.12	1.16	0.441

the yield of cheese from clam rennet was approximately 0.17% lower than from calf rennet. Cheese yield may be influenced by fat loss, solids-non-fat loss and loss of curd fines (Tofte-Jespersen and Dinesen, 1979). A distinct relation between fat loss and type of coagulant used has not been reported in detail to any extent. However, loss of solids-non-fat was highly influenced by the proteolytic activity carried out by the coagulant used (Emmons and Beckett, 1977). Thus, the reduced yield of cheese prepared with clam rennet was probably caused by the coagulant's high proteolytic activity. This was consistent with the high levels of total and nonprotein nitrogen in whey (Table 3). The high level of nonprotein nitrogen in clam rennet whey was partly attributable to the crude coagulant preparation, which contained 10 mM cysteine used to activate the cathepsin B-like enzyme. This amount of nitrogen contributed by clam rennet was also detectable in the milk which was just renneted. The increase in NPN for calf rennet was 0.024 (0.268–0.292) whereas it was 0.145 (0.296–0.441) for clam rennet. However, the differences between total and nonprotein nitrogens in whey samples were similar for both coagulants, namely 0.728 for calf rennet and 0.719 for clam rennet. These facts imply that clam rennet has low activity toward whey proteins, as does calf rennet, but was approximately 6 times more proteolytic than calf rennet towards casein following renetting. In commercial practice, the average level of total protein nitrogen in whey is 0.7–0.8% (Kosikowski, 1977). High levels of total nitrogen in both whey samples reflected the relatively larger loss of curd fines from the small-scale cheese-making done in this study. The data should not be viewed as conclusive as more batches of cheese need to be made to obtain statistically relevant results regarding the yield and composition of cheese made with clam rennet.

Cheese ripening and sensory evaluation. Traditional methods for following proteolysis during ripening of cheese depend upon measuring the increase of various forms of soluble nitrogen. However, it was believed that total tyrosine liberated in aging cheese was a more sensitive criterion of ripening that the soluble nitrogen content (Silverman and Kosikowski, 1955). Flavor intensity was estimated by professional judges also showed a linear relationship to soluble tyrosine (Vakaleris and Price, 1959). The amounts of soluble nitrogen, tyrosine and tryptophan in the extracts from both clam rennet cheese and calf rennet cheese of different ages are shown in Table 4. Clam rennet cheese was ripening much faster than calf rennet cheese as indicated by all three parameters. The levels of soluble nitrogen, tyrosine and tryptophan in 2-wk-old clam rennet cheese were comparable to those of 8-week-old calf rennet cheese. In 10-wk-old clam rennet cheese, these levels were compatible to those of 5 to 6-month-old calf rennet cheese made by other investigators (Vakaleris and Price, 1959; Shamsuzzaman and Haard, 1983).

Table 4—Soluble nitrogen, tyrosine (Tyr) and tryptophan (Trp) contents of cheese extracts

Age (wk)	Clam rennet cheese: Soluble N ($A_{274.5}$)	Tyr (mM)	Trp (mM)	Calf rennet cheese: Soluble N ($A_{274.5}$)	Tyr (mM)	Trp (mM)
2	0.798	0.186	0.115	0.480	0.085	0.075
4	0.894	0.251	0.115	0.548	0.120	0.080
6	1.068	0.290	0.138	0.654	0.148	0.094
8	1.238	0.349	0.155	0.762	0.167	0.110
10	1.504	0.435	0.184	0.888	0.251	0.114

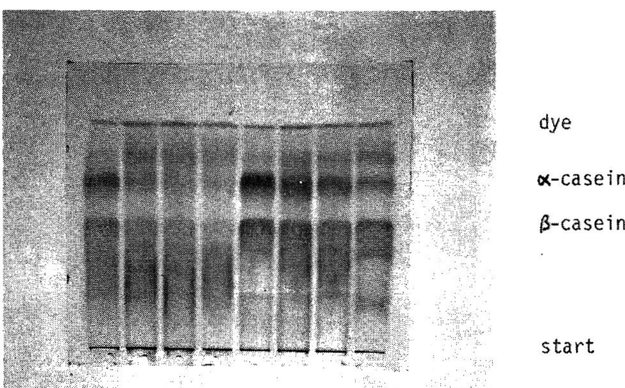

Fig. 7 — The electrophoretic patterns of clam and calf cheeses. Lanes 1-4 are from 2, 4, 6 and 8-wk-old clam cheese, respectively. Lanes 5-8 are from 2, 4, 6 and 8-wk-old calf cheese, respectively.

Electrophoresis patterns of cheese have been used to compare the proteolytic action of milk coagulants on caseins (Mickelsen and Fish, 1970; Vanderpoorten and Weckx, 1972). The electrophoretic patterns of cheeses at different ripening stages are shown in Fig. 7. More proteolysis was obvious in clam rennet cheese than calf rennet cheese as indicated by the decline in color intensity of alpha- and beta-caseins with age in two different cheeses. The color intensity of alpha- and beta-caseins in 2-wk-old clam rennet cheese was similar to 8-wk-old calf rennet cheese. The result was consistent with the soluble nitrogen, tyrosine and tryptophan contents. In calf rennet cheese alpha-casein degradation proceed faster than beta-casein. Both caseins were degraded very fast by clam rennet. The degradation of alpha-casein in cheese made from microbial rennet tends to be relatively slow as compared with beta-casein (Vanderpoorten and Weckx, 1972).

The densitometer scanning of electrophoretic patterns of proteins from 2-wk-old clam rennet cheese and 8 week-old calf rennet cheese is shown in Fig. 8. Pattern similarity appeared to exist between these two samples. Although protein degradation is not the only contributing factor to the aging of cheddar cheese, it is expected that the cheeses which are sensorily compatible should have similar electrophoresis (or gel filtration) patterns. The preference scores for 10-week-old cheeses are shown in Table 5. None of the pairs of treatments differed significantly ($P > 0.10$) for any of the three attributes.

The extent of incorporation of active coagulant into cheese has been shown to be a function of the particular coagulant used (Holmes and Ernstrom, 1973). In general, about 2–3% of the coagulant is contained in the curd as an active enzyme after processing, and it is this residual enzyme that assists in the ripening process. A current hypothesis for the process of flavor development is that a low redox potential has to be achieved within the cheese to allow chemical reactions to take place which result in the production of flavor components such a methanethiol (Manning, 1979). Microbial growth brings about a condition of low redox potential, and the more rapid and

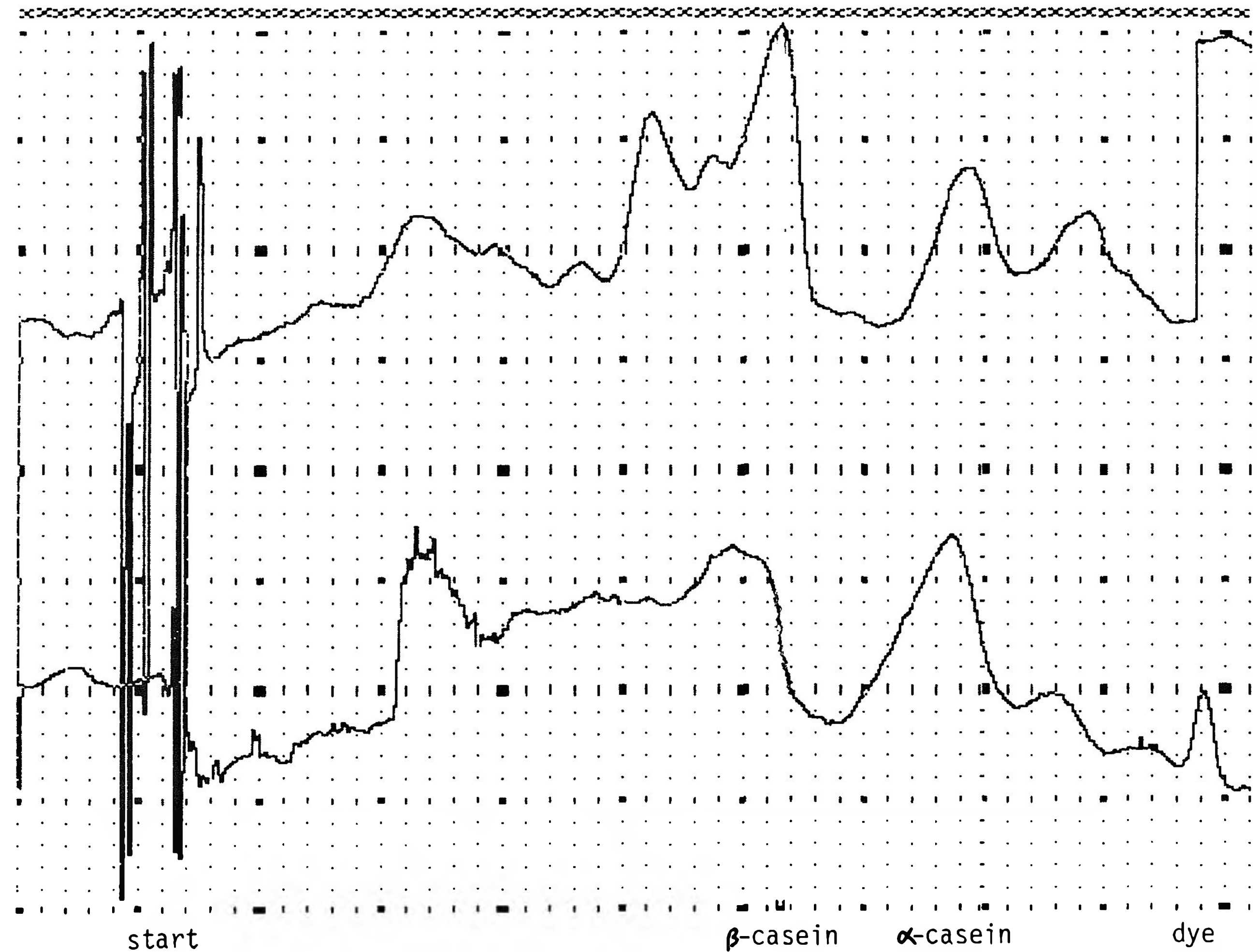

Fig. 8—Densitometer scanning and electrophoretic patterns of 2-week-old clam cheese (bottom) and 8-week-old calf cheese (top).

Table 5—Sensory evaluation scores[a] of 10-week-old cheeses

Samples	Flavor	Texture and Body	Acceptability
Calf rennet-1	6.90	7.00	6.90
Calf rennet-2	7.00	6.90	6.90
Clam rennet-1	6.90	6.90	6.90
Clam rennet-2	7.30	7.10	7.30

[a] 9 = like extremely and 1 = dislike extremely

vigorous the growth, the quicker the ripening and flavor development are likely to be. Milk coagulants, by hydrolyzing some of the peptide bonds, degrades casein proteins to more assimilable fractions and thus encourage bacterial growth. As clam rennet is more proteolytic and has broader specificity than calf rennet, it is likely to produce more assimilable peptides to encourage microbial growth and accelerate the ripening process.

The choice of starter culture is important in flavor development. When large amounts of calf rennet were used to give increase proteolysis, fast acid starters produced bitter peptides while slow acid starters did not (Lawrence et al, 1972; Lowrie and Lawrence, 1972). It would be of interest and value to further investigate the effect of different starter cultures and/or coagulant amounts on the quality of cheese made from clam rennet. In addition, the use of a mixture of clam rennet and other high clotting power enzyme, such as calf rennet or porcine pepsin, may prove to be more beneficial to the cheese quality or more economically feasible.

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N-Nitrosothiazolidine and N-Nitrosothiazolidine-4-Carboxylic Acid in Smoked Meats and Fish

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ABSTRACT

Various smoked meats including raw and fried bacon and both unsmoked and smoked (uncured) fish were analyzed for NThZ and NTCA. Most meat samples, bacons, and a few smoked fish contained traces of NThZ and considerably high (up to 13,700 ppb) levels of NTCA. Preliminary results indicated that raw bacons processed by old-fashioned direct smoking methods contained the highest (>1,000 ppb) level of NTCA. Formation of NThZ from the NTCA present in smoked bacon increased with an increase in frying temperature as well as with frying time. NTCA level in the raw bacon and the cooking conditions seemed to be the most important factors involved in the formation of NThZ in the fried bacon. The reason for finding only insignificant levels of NThZ in smoked fish could not be established conclusively.

INTRODUCTION

N-NITROSOTHIAZOLIDINE (NThZ) and N-trosothiazolidine-4-carboxylic Acid (NTCA) are two newly discovered N-nitroso compounds which have been found to occur consistently in some smoked foods, mainly cured meats and bacon (Kimoto et al., 1982; Pensabene and Fiddler, 1983a; Sen et al., 1985a; Mandagere et al., 1984; Helgason et al., 1984). Although most of the N-nitroso compounds tested, thus far, have been shown to be carcinogenic in laboratory animals (Preussmann and Stewart, 1984) very little is known about the toxicity of NThZ or NTCA. Until now, most of the research on these two compounds has been limited to their analysis, occurrence, and formation in foods. A recent study by Loury et al. (1984), however, has indicated genotoxicity of NThZ in microbial and hepatocellular test systems. NTCA, on the other hand, has been shown to induce diabetes in experimental animals, and has been implicated as the causative agent for the high incidence of diabetes in juveniles whose mothers consumed high NTCA-containing smoked meats during pregnancy (Helgason et al., 1984).

Studies by Pensabene and Fiddler (1983b) suggested that NThZ in raw bacon was mainly formed during the heating-smoking step and, unlike N-nitrosopyrrolidine (NPYR), its concentration in raw bacon was higher than that in the fried product. Based on these findings these researchers postulated that NThZ in fried bacon originated from that present in the raw bacon and none was formed during frying. Sen et al. (1985a), on the other hand, noted that while the above conclusion might be true in some cases, considerable amounts of NThZ could form during frying of other bacon samples. The levels of NThZ in the fried products in those cases were higher than that detected in the corresponding raw bacons. For example, one fried bacon contained as high as 241 ppb NThZ whereas the raw bacon contained only 7.5 ppb. The latter workers (Sen et al., 1985a) also demonstrated conversion (due to heat induced decarboxylation) of NTCA to NThZ under actual bacon frying conditions. Their preliminary data indicated a good correlation of NTCA levels in raw bacons with NThZ levels in the fried products. Recent unpublished data by Mandagere et al. (1984) (see Skrypec et al., 1985) are consistent with the above findings.

Differential scanning calorimetric studies with NTCA have shown (Mandagere et al., 1984) that it can easily decarboxylate to NThZ at 180°C—a temperature that is easily attained during frying of bacon. The frying temperature is, however, much higher than the processing temperature of raw bacon, thus explaining the lack of correlation between NTCA and NThZ levels in raw bacon (Sen et al., 1985a). NThZ in raw bacon and other smoked meats is probably formed by nitrosation of thiazolidine (Kimoto et al., 1982; Mandagere et al., 1984; Sen et al., 1985a). Figure 1 summarizes various possible reactions that may lead to the formation of NThZ and NTCA. The nitrosating agent mainly originates from the added nitrite and to a smaller extent from NO_x gases in the smoke (if directly smoked), and the source of the HCHO is believed to be the smoke although a small amount may also come from sugar (Skrypec et al., 1985; Pensabene and Fiddler, 1983b; Tricker et al., 1984).

Although it has been clearly established that both NThZ and NTCA can occur in various smoked meats and fish, there appear to be some uncertainties as to both their levels in various foods as well as to their mode of formation. For example, it is not clear why some raw bacons contain more NTCA than others. The discrepancy as to the relative levels of NThZ in raw versus fried bacon is still to be fully resolved. The present study was undertaken to obtain additional data on NThZ and NTCA levels in raw and fried bacon and smoked fish, and to further study the mechanism of formation in foods.

MATERIALS & METHODS

Samples

Most of the samples were either purchased in the local retail outlets or picked up directly from the plants by Health Protection Branch inspectors. Each sample of meat or fish was cut into small pieces, homogenized well using a blender, and stored at 4°C in a glass jar. They were analyzed usually within a week. Alternate slices of raw bacon were fried within 1 or 2 days of procurement or arrival (shipped with dry ice) at the laboratory. The remaining slices were homogenized and analyzed as raw product. All four liquid smokes used in the study were obtained from various meat packers.

In total, 32 samples of various fish were procured for analysis. They were as follows: seven samples of various unsmoked (one each of salted cod, salted herring, frozen sole, frozen cod, fresh salmon, and two canned tuna) and 25 smoked fish and seafoods (one each of mackerel, cod roe and liver, shellfish, mussels, oysters, haddock, sardine; two kippers; three rainbow trout; four each of Alaskan cod, salmon; and five herring).

Smoking method

Information about different smoking methods was gathered through the courtesy of the Health Protection Branch inspectors. Basically, two types of direct smoking processes were used. In the modern method, smoke generated by burning maple wood sawdust was filtered through water before it was allowed to enter in the smoking room which housed the bacon slabs. The smoke was circulated through the room with controlled moisture for 4–24 hr (final temperature 58–70°C). In the old-fashioned direct smoking method smoke was produced in the same manner as above but it was allowed to enter the smoking chamber without filtering through water. Also, there was no forced air or

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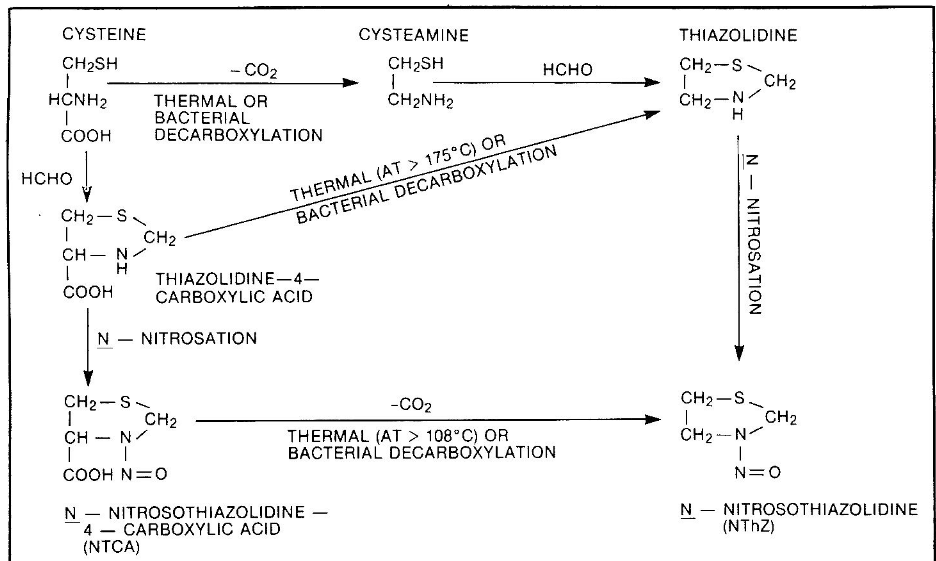

Fig. 1—Possible pathways for the formation of NThZ and NTCA in foods (bacterial decarboxylation steps have not yet been demonstrated).

agitator, and even the moisture level was not controlled. Some of the bacon samples from the retail outlets were processed with liquid smoke, but the details of the procedure were not available.

Frying procedure

Bacon slices were fried using a Teflon-coated electric frypan (starting from a cold pan) as described previously (Sen et al., 1979). The pan was precalibrated by heating cooking oil and checking the maximum temperature attained under different thermostat settings. The actual temperatures reached at various settings were as follows: (A) 10 min at 165° setting, 177°C; (B) 10 min at 190°C setting, 190°C; (C) 8 min at 175°C setting, 180°C; and (D) 14 min at 175°C, 180°C. The resulting fried bacon in most cases was crisp but not burnt.

The fried bacons were homogenized (after freezing on dry ice), and stored as described previously (Sen et al., 1985a).

Incubation studies

To determine the limiting precursors (nitrite, cysteamine, HCHO) responsible for the formation of NThZ in the respective smoked foods or liquid smoke, 10–20g of a sample were mixed well in a beaker with 10 mL water and 1 mL solutions of appropriate amounts of the precursors (HCHO, 37 mg/mL; cysteamine HCl, 100 mg/mL; $NaNO_2$, 50 or 500 μg/mL). The beaker was covered with aluminum foil, and the mixture allowed to sit overnight at 20°C. The mixture was then analyzed for NThZ as described previously (Sen et al., 1985a).

Determination of NThZ and NTCA

The details of the methods have previously been reported (Sen et al., 1985a). The only change made in the method was that prior to vacuum distillation of fish samples, 4g $Ba(OH)_2$ were added (in addition to 3N KOH) to the sample to minimize foaming. The method has a detection limit of about 1 ppb for a 20-g sample.

During the analysis of meat and fish samples for NTCA, *N*-nitrosopipecolic (NPIC) acid was routinely added to each sample at the start of the analysis to monitor the efficiency of the overall analytical process. The results, however, were not corrected for percentage recoveries (average, 90%) of NPIC. In cases when the final extract was too dirty (highly colored) it was cleaned-up on a silica gel Sep-Pak (Water Associates, Milford, MA) cartridge as described previously (Sen et al., 1985a). The detection limit of the method was about 4–10 ppb.

In a few selected cases, the identity of NThZ and NTCA was confirmed by GLC-high resolution mass spectrometry (selected ion monitoring at a resolution of 4–6.5k) as described previously (Sen et al., 1985a). Ions monitored were as follows: NThZ, M^+ and $(M-NO)^+$; NTCA, at m/z 117 $(M\text{-}COOCH_3)^+$, m/z 118 $(M-NO-CO)^+$, and m/z 146 $(M-NO)^+$.

Determination of nitrite

A 10-g aliquot of the homogenized meats or raw bacons was analyzed for residual nitrite by the method of Sen and Donaldson (1978).

RESULTS & DISCUSSION

TABLE 1 gives a summary of the results obtained in this study. The data confirm previously published results (Pensabene and Fiddler, 1983a; Skrypec et al., 1985; Sen et al., 1985a) in the sense that most smoked meats contain low levels of NThZ and most smoked fish are negative for NThZ (Sen et al., 1985b). However, the average (omitting the outliers) NThZ level in fried bacon as reported here appears to be higher (19.4 ppb) than we reported previously (6.3 ppb; Sen et al., 1985a). The reason for this difference is discussed later.

We failed to observe a correlation between residual nitrite (Tables 2 and 4) and NThZ levels in the raw bacon and other smoked meats. Similar results were also reported by Pensabene and Fiddler (1983b). This suggests that nitrite concentration is probably not the limiting factor in the formation of NThZ. This may be because most cured meats are processed with up to 150–200 ppm sodium nitrite (Canadian Food and Drugs Act and Regulations, 1981) and, therefore, there is an excess of nitrite present from the beginning. None of the four liquid smokes analyzed contained NThZ; this is consistent with the findings of Mandagere et al. 1984).

In the incubation experiments with smoked meats and raw bacon, the addition of both cysteamine and HCHO were required for the formation of substantial amounts of NThZ (Table 2). Except in one sample, the addition of either compound alone did not increase the NThZ levels, suggesting that neither of the two precursors was present in excess. Only in one case (a pepperette) was there a considerable increase (6.5 ppb to 39 ppb) in NThZ formation after incubation with cysteamine alone. This sample was processed in a direct smoking, old-fashioned smoke-house which may have contributed excess HCHO. Since nitrite was already present in these samples and since the rate of nitrosation of thiazolidine (formed easily from cysteamine and HCHO) is very fast (Pensabene and Fiddler, 1982), the most likely limiting precursors would be expected to be either cysteamine or HCHO or both compounds. Therefore, the results of the incubation experiments are consistent with the above reasoning. Before any definite conclusions is reached, however, actual levels of HCHO, cysteamine, and thiazolidine in such products should be determined.

Similar incubation studies (Table 3) indicated that the liquid smokes probably contained only low levels of HCHO because incubation with cysteamine and nitrite (no meat used) increased their NThZ contents only slightly. Had there been substantial amounts of HCHO present, one would have observed formation of higher levels of NThZ (as was observed when liquid smokes were incubated with all three precursors). This

Table 1—NThZ and NTCA levels in various smoked meats and fish

Item	No. of samples analyzed	NThZ levels (ppb)		NTCA levels (ppb)	
		Mean	Range	Mean	Range
Miscellaneous smoked meats (ham, sausage, bologna, frankfurter, pepperoni, slow-cured dry sausage)	15	4.3	N[a]-11	266[b]	N-3,900
Raw bacon	12	6.3	1.5-14.4	1,800	N-9,000
Fried bacon (levels based on the weight of the fried sample)	11	19.4[c]	4.7-241	1,615[d]	100-13,700
Fish and seafoods	32	<1	N-1.8	67[e]	N-1,600

[a] N = negative (detection limit, 1 ppb for NThZ and 4-10 ppb for NTCA).
[b] Excluding one sausage (smoked in an old-fashioned smokehouse) and one pepperettes (made by a family operated small producer) which contained, respectively, 3,900 ppb and 2,100 ppb NTCA.
[c] Excluding two samples containing 100 ppb and 241 ppb NThZ.
[d] Excluding two fried bacons each containing ≈14,000 ppb NTCA.
[e] Excluding one smokeed herring which contained 1,600 ppb NTCA; only a total of 20 samples were analyzed for NTCA.

Table 2—Effect of incubation with various precursors of NThZ on its formation in smoked meats and raw bacon[a]

Sample	Residual nitrite (as $NaNO_2$) ppm	NThZ level (ppb) formed after incubation with				
		None	Cysteamine	HCHO	Cysteamine + HCHO	HCHO + Nitrite
Ham	118	2.2	2.3	–[c]	—	—
Ham	24	N[b]	N	N	27	—
Ham	36	10.5	10.5	9.4	—	—
Dry salami	7	3.2	3.3	3.5	—	5.4
Pepperoni	56	N	N	N	—	N
Beef sticks	13	3.3	3.8	4.7	—	3.5
Pepperettes (old-fashioned smoking)	48	6.5	39.0	7.6	—	—
Sausage	15	1.2	1.5	1.2	—	4.0
Deli-stick	12	3.5	2.4	2.7	—	—
Bacon	68	1.5	2	N	27.6	—
Bacon	86	1.7	1.2	N	84	—
Bacon	16	4.3	4.6	6	3,200[d]	—

[a] Aliquots (10–20g) of samples were incubated with 10 mL water and 1 mL solution of appropriate precursors (HCHO, 37 mg/mL; cysteamine HC1, 100 mg/mL; $NaNO_2$ 500 μg/mL)
[b] N = negative (<1 ppb).
[c] Not carried out.
[d] This sample was incubated for 16 hr at room temperature and then for an additional 48 hr (weekend) at 4°C.

Table 3—Formation of NThZ during incubation of liquid smoke and known precursors of NThZ

Sample	NThZ level (ppb) formed after incubation with [a]				
	None (control)	Nitrite	HCHO + Nitrite	Cysteamine + Nitrite	HCHO + Nitrite + cysteamine
A	N[b]	N	N	10.8 (225)	378
B	N	N	N	(289)	–[c]
C	N	N	N	Traces	2,800
D	N	N	N	3.4	197

[a] 50 μg $NaNO_2$ was used per test in all cases except in those in parentheses where 500 μg $NaNO_2$ was used.
[b] N = negative (<1 ppb).
[c] Not carried out.

may explain why Pensabene and Fiddler (1983b) observed only traces (avg 1.3 ppb) of NThZ in bacons processed with liquid smoke whereas those processed by direct smoking contained much higher (avg 5.5 ppb) levels of NThZ. It is possible that during manufacture of the liquid smokes, some of the HCHO had been removed. The differences in the method of producing liquid smokes as well as batch to batch variations might also be responsible for some of the differences observed. Also, the wide variations in results for the formation of NThZ from all three precursors suggested that some of the liquid smoke may have contained *N*-nitrosation inhibitors such as phenol and syringol (Issenberg and Virk, 1974). Studies are in progress to determine the actual concentration of such phenols in the various liquid smoke samples used in this study.

Table 4 gives the detailed results of nitrite, NThZ, and NTCA levels in various raw and fried bacons analyzed in this study. The data indicated a positive correlation between NTCA levels in raw bacon and the NThZ content of fried bacon, but not with that of raw bacon, thus confirming our previous findings (Sen et al., 1985a). All raw bacons containing >1,000 ppb NTCA formed >30 ppb NThZ upon frying. The data on the effect of variation in frying conditions (Table 4) indicated that higher frying temperatures and longer frying times produced higher levels of NThZ in the fried product. This observation along with the observed correlation between NTCA levels in raw with NThZ levels in fried bacon are consistent with the theory of formation of NThZ by heat induced decarboxylation of NTCA as proposed by us previously (Sen et al., 1985a). At least in these bacons, NTCA appears to be the main precursor of NThZ detected in the corresponding fried samples.

Furthermore, most fried bacons contained higher levels of NThZ than the corresponding raw bacons — a finding opposite to that reported by Pensabene and Fiddler (1983b). It is possible that the raw bacons they analyzed had lower NTCA contents. The difference in the methods of frying and analysis (for NThZ) in the two studies may also be responsible for this discrepancy.

The lack of correlation between NTCA and NThZ levels in raw bacon can be attributed to the fact that the normal smoking and processing temperature for producing bacon is not high enough to decarboyxlate NTCA to NThZ (Skrypec et al., 1985). NThZ in raw bacon is probably formed by direct nitrosation of thiazolidine. The role, if any, of bacterial decarboxylation (Fig. 1), which may occur under milder temperature, in the formation of NThZ in raw bacon and other smoked meat products is yet to be investigated.

Our preliminary data (Table 4) indicated that most bacons processed by old-fashioned direct smoking process contained the highest level of NTCA, whereas those processed with liq-

Table 4—Levels of nitrite, NThZ and NTCA in various samples of smoked raw and fried bacon

	Sample and smoking process	Raw			Fried	
		Nitrie, ppm as $NaNO_2$	NThZ ppb	NTCA ppb	NThZ, ppb (frying condition)[a]	NTCA, ppb (frying condition)
(1)	Bacon from retail outlets (liquid smoke)	–[b]	14.4	328	9.7(A)[a]; 16(B)	218 (A)
(2)	Bacon from retail outlets	74	3.9	135	9(A); 14(B)	264 (A)
(3)	Bacon from retail outlets	—	2.3	35	6.7(A)	—
(4)	Bacon from retail outlets (direct smoking)	68	1.5	N[e]	8.4(A)	100 (A)
(5)	Bacon from retail outlets (liquid smoke)	86	1.7	N	4.7(A)	168 (A)
(6)	Bacon processed with liquid smoke	40	4.5	36	6.5(A)	403 (A)
(7)	Brine injected, smoked in an old-fashioned smokehouse; Producer, 'J'	16	4.3	3,200[c]	37(C); 155[c](D)	2,200[c](C)
(8)	Brine injected, smoked in an old-fashioned smoke-house; Producer, 'K'	36	7.3	5,400[c]	69[c](C); 275(D)	13,700 (C)
(9)	Brine injected, smoked in an old-fashioned, smoke-house; Producer, 'K'	57	9.7	2,700	100 (C); 654[c](D)	1,500 (C)
(10)	Brine injected, smoked in a modern smokehouse[d]; Producer, 'J'	47	3.2	1,400[c]	4(C); 37(D)	1,100 (C)
(11)	Smoked in an old-fashioned smokehouse	—	7.5	9,000	241(A)	14,000 (A)
(12)	Injected with brine containing liquid smoke, heated and smoked in an old-fashioned smokehouse, Producer, 'L'	16	4.3	306	8.2(A)	2,000
(13)	Processed with dry cure, then smoked in an old-fashioned[d] smokehouse; Producer, 'K'	12	4.1	4,300[c]	36(C); 138[c](D)	2,800 (C)
(14)	Processed with dry cure, then smoked in an old-fashioned[d] smokehouse; Producer, 'K'	12	3.3	3,800[c]	42[c](C); 135(D)	5,800[c](C)

[a] Capital letters in parentheses represent frying conditions. All samples were fried starting from a cold pan: (A) 20°C — 177°C, 10 min total; (B) 20°C — 190°C, 10 min total; (C) 20°C — 180°C, 8 min total; and (D) 20°C — 180°C, 14 min total.
[b] Not analyzed.
[c] Confirmed by GLC-high resolution mass spectrometry.
[d] Direct smoking was used in both cases. In the modern method smoke was filtered through water before it was allowed to enter in the smoke house whereas in the old-fashioned method smoking was done using raw (unwashed) smoke.
[e] N = negative (detection limit, 1 ppb for NThZ and 4–10 ppb for NTCA).

uid smoke contained either undetectable or extremely low levels of NTCA. Smoke generated by the former process probably contributed higher levels of HCHO than when liquid smoke was used. However, there was an exception. A sample of bacon (No. 4, Table 4) processed by direct smoking NTCA. It is possible that this sample lacked the necessary amine precursor namely, free cysteine, or maybe the smoking period was too brief to contribute sufficient amounts of HCHO. Other factors (catalysts, meat composition, curing conditions, etc.) may also be important in the formation of NTCA. Therefore, a much more extensive study should be carried out before reaching any definite conclusion.

Pensabene and Fiddler (1985) noted that spraying pork bellies with liquid smoke before or during processing significantly inhibited NThZ formation in raw bacon. Similar situations might have existed in sample No. 12 (Table 4) which was the only sample that had been treated with liquid smoke before being smoked further in a smokehouse. This sample also contained only 306 ppb NTCA despite the smokehouse treatment. It should also be pointed out that the average (after omitting the outliers) levels of NTCA found in raw and fried bacons were much higher than those reported by us in a previous study (Sen et al., 1985a). This is probably because of a biased selection of samples that were smoked in old-fashioned smokehouses. Since these samples contained higher levels of NTCA, the corresponding fried bacons contained higher average levels of NThZ than that detected previously.

Most of the fish and seafoods analyzed were negative for NThZ (Table 1); only 3 (one each of smoked oyster, smoked herring and smoked trout) contained traces (~1 ppb). When some of these samples (Table 5) were incubated with nitrite or with a mixture of nitrite and HCHO there was no detectable increase in the NThZ formation, whereas incubation with a mixture of cysteamine and nitrite increased the NThZ content appreciably (from ~1 ppb to 7 ppb). As expected, incubation of two fish samples with all three precursors increased their NThZ contents (up to 360 ppb); these two experiments served as a positive control. The most unexpected result came from the experiments in which the fish samples were incubated with a mixture of HCHO and cysteamine. In two out of four cases, the NThZ contents increased considerably (to 55 ppb and 305 ppb) thus suggesting the presence of traces of nitrite or other nitrosating agents in these two samples. Since nitrosation rate of thiazolidine is very fast, even traces of nitrite might be sufficient to form significant amounts of NThZ if the other precursors are present in excess.

Previously, we (Sen et al., 1985b) suggested that the lack of formation of NThZ in smoked fish might be due to absence of nitrite which is not permitted as an additive in fish in Canada (Canadian Food and Drug Regulations, 1981). The above data from the incubation experiments (Table 5) indicate that the lack of formation of NThZ in smoked fish might not be entirely due to the absence of nitrite; the precursos HCHO and cysteamine might also be absent. Alternatively, it is possible that nitrite needs to be present at the beginning of the smoking process when the formation of NThZ takes place. Since no

Table 5—Incubation of smoked fish with various precursors of NThZ

Sample	$NaNO_2$ ppm	NThZ level (ppb) present or formed after incubation with[a] None	Nitrite (500 μg)	Cysteamine + Nitrite (500 μg)	HCHO + Nitrite (500 μg)	HCHO + Cysteamine	HCHO + Cysteamine + Nitrite (50 μg)
Smoked cod	N[b]	N[b]	N	N	N	3.5	117
Smoked kipper	N	N	N	0.8	N	3.1	360
Smoked salmon	N	N	N	6.9	1.0	55	—[c]
Smoked trout	0.7	1.4	0.7	6.9	1.5	305	—

[a] For details, see text.
[b] N = Negative (<0.5 ppm for $NaNO_2$ and <1 ppb for NThZ).
[c] Nct carried out.

nitrite-cured smoked fish are available in Canada we were unable to test this hypothesis.

Eight of the 20 smoked fish analyzed contained low levels of NTCA. One sample (a smoked herring) contained an extremely high (1,600 ppb) level of NTCA. Nitrogen oxide gases present in smoke, or nitrite, produced (as a result of microbial reduction) from nitrates in water or present in salt (as impurity), are possible sources of the nitrosating agent. Free cysteine of other amino acids present in these protein-rich foods may have consumed all the HCHO and nitrosating agents thus leaving very little for the formation of NThZ via thiazolidine (Fig. 1).

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Color Changes and Available Lysine during Storage of Shelf-Stable Concentrated Cheese Whey

ROSA J. DE KANTEREWICZ and JORGE CHIRIFE

ABSTRACT

Concentrated whey (about 50% solids) was stabilized against microbial deterioration by a combination of a slight reduction of water activity (a_w) (0.94 or 0.92), lowered pH (5.2 or 5.4) and addition of 0.2% potassium sorbate. Color changes and available lysine loss in the above shelf stable whey, stored at different temperatures, were studied. A zero order reaction kinetics was observed for color changes during browning of the concentrated whey; activation energy was found to be 26 Kcal/mole. The loss of available lysine amounted to 20–30% of the initial value after three months of storage at 30°C.

INTRODUCTION

VERY LARGE AMOUNTS of whey are produced all around the world; however, only a small percentage of this whey is utilized or processed, the rest merely disposed of by any available means. Kanterewicz et al. (1986) recently developed a simple method to achieve microbial stability of concentrated whey (about 50% whey solids) during nonrefrigerated storage. They found that this concentrated whey may be stabilized by a combination of a slight reduction of water activity (a_w), lowered pH and addition of potassium sorbate. The whey so obtained may be stored for at least three months at 30°C without bacterial or mold deterioration. This finding may open new possibilities for a better utilization of this valuable by-product of the dairy industry as an inexpensive and nutritious alternative ingredient in food products. For this reason, in addition to the microbial stability, the chemical stability of whey is also of much importance. Nonenzymatic browning via the Maillard reaction is one important node of deterioration in whey products which may limit their shelf life (Saltmarch et al., 1981; Labuza and Saltmarch, 1981a).

Concentrated whey contains relatively high concentrations of lactose and protein high in lysine; thus, in the presence of moisture, these components may readily participate in the Maillard reaction (Finot et al., 1981; Labuza and Saltmarch,1981 a). A decrease in the quality of protein may result as a consequence of this interaction and undesirable color changes may also occur.

This study was concerned with color changes and protein quality loss in shelf stable concentrated sweet whey stored at different temperatures.

MATERIALS & METHODS

Materials and sample preparation

Concentrated sweet whey was supplied by SANCOR Cooperatives Unidas Ltda., Buenos Aires; it was obtained by concentrating liquid whey (solids 6–7%; lactose 4.6–4.8%; proteins 0.8–0.9%; fat 0.03%; ash 0.5%) in a falling film evaporator at 70°C until the solution contained about 50% solids. The water activity of this concentrated whey was 0.94 and it was further lowered by addition of the appropriate amount of sodium chloride.

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The water activity of the samples was determined using a LUFFT fiber-dimensional hygrometer. Citric acid was used to reduce pH to the desired value. As shown previously (Kanterewicz et al., 1985) microbial stability in concentrated whey incubated at 30°C may be achieved by adjusting a_w, pH and potassium sorbate to two combinations, namely, a_w 0.94 - pH 5.2 - 0.2% sorbate, or a_w 0.92 - pH 5.4 - 0.2% sorbate. For this reason these two combinations were selected to study color and protein quality changes during nonrefrigerated storage. The samples of concentrated whey were held at constant temperature in an air-circulating oven at 30°C, 38°C, and 45°C.

Color measurement

It is well known that the optical density of browned solutions at a particular wavelength is the variable most frequently used to measure the progress of nonenzymatic browning reactions in foods. However, this may not always be the most reliable way to describe the visual color changes in browned food systems (Petriella et al., 1985). For this reason a spectrophotometric tristimulus color measurement of heated whey samples was performed. Color measurements were taken with an automatic recording spectrophotometer ZEISS DMC 25. The spectrophotometric reflection curves were obtained and the CIE tristimulus values were calculated directly in the instrument by means of an automatic integrator (Davidson and Hemmendinger Electronic, Tatamy, PA). All measurements were taken using 10-mm glass cells, under CIE illuminant C with $BaSO_4$ as reference standard. Reflectance readings were converted to the corresponding values for several other color functions in the CIELUV and CIELAB color spaces. The metric saturation S_{uv} (CIELUV), metric chroma C^*_{ab} (CIELAB) and the luminosity L^* were chosen as the most suitable functions (CIE, 1976).

Available lysine

Chemically available lysine (ε-DNP-lysine) was determined using the Booth (1971) modification of the Carpenter (1960) method.

RESULTS & DISCUSSION

Color changes

The color functions (S_{uv}, C^*_{ab}, and L^*) were calculated through the equations for the CIELUV and CIELAB color space, as reported by Petriella et al. (1985). Figure 1 shows a plot of the different color functions — metric saturation (S_{uv}), metric chroma (C^*_{ab}), and luminosity (L^*) — as a function of heating time at 45°C for a shelf-stable concentrated whey having $a_w = 0.94$ and pH 5.2.

The differences values of the various color functions were plotted, i.e., $(S - S_o)_{uv}$, $(C^* - C_o)_{ab}$, and $(L_o - L^*)$, with S_o, C_o and L_o the values corresponding to the whey samples without storage. It can be seen that all three color functions show similar behavior, i.e., they are a linear function of time (correlation coefficient are 0.9969, 0.9863 and 0.9928, respectively). It follows that the simplest model for prediction of non-enzymatic browning of the shelf stable whey is that of zero order reaction, were

$$\frac{dB}{d\theta} = k_B = \text{rate of browning}$$

where B may be expressed in terms of any of the color functions S_{uv}, C^*_{ab} or L. A zero order reaction kinetics for color changes during browning of a glucose-lysine model system of a_w and pH similar to that of whey has been recently reported

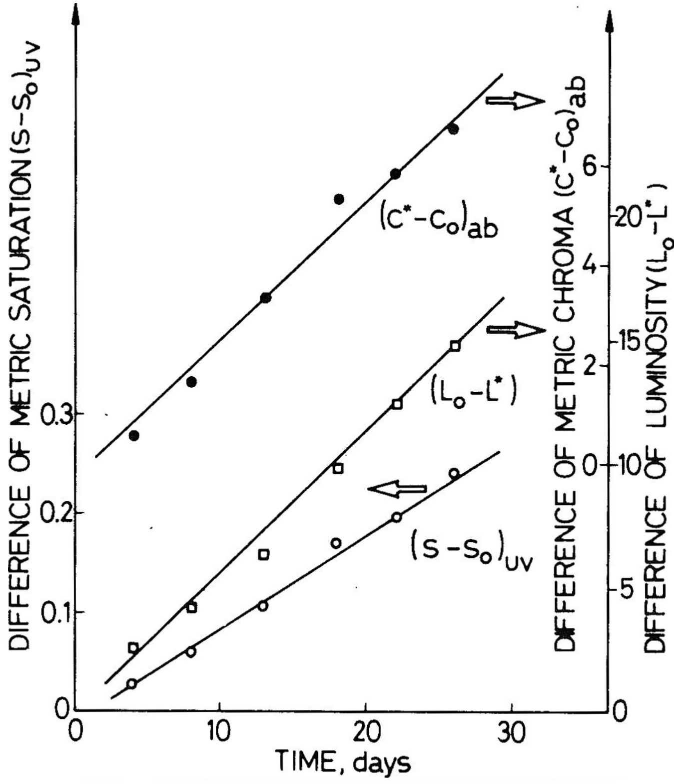

Fig. 1—Effect of heating time at 45°C on the increase of different color functions for shelf stable whey at a_w = 0.94 and pH = 5.2.

by Petriella et al. (1985). Labuza and Saltmarch (1981a) also found that brown pigment formation (as obtained from measurements of optical density) in stored whey powder having a_w in the range 0.33–0.65 also followed a zero order reaction kinetics.

Figure 2 shows color development (as measured by changes in L*) in shelf stable whey samples stored at 30°C, 38°C and 45°C. The great influence of temperature on the rate of color development was apparent. After 60 days of storage at 30°C (corresponding to $(L_o - L^*) \cong 4.1$), the color of whey samples was quite acceptable from commercial standpoint, as judged by visual inspection of the samples.

Rate constant $k^{(S-S_o)}$ and $k^{(L_o-L^*)}$ for color development in shelf stable whey samples are listed in Table 1; they were obtained by linear regression analysis of the linear plots of measured color functions versus time for each temperature. It can be seen that the relative effect of the process variables was similar for both color functions, S_{uv} and L*. The rate of color development in both whey samples (a_w 0.94, pH 5.2 and a_w 0.92, pH 5.4) was quite similar, although for all cases studied it was slightly less for the sample of higher a_w-lower pH. For all cases examined *r* values for rate constants were greater than 0.98; for most runs the ± 95% confidence limits were calculated to be between ± 8 to ± 20% of the value. These figures are acceptable if one considers the inherent variability of a real food such as the concentrated whey studied here.

The activation energy, E_a, obtained from the slope of Arrhenius plots was quite similar for the two different shelf stable whey samples (a_w 0.94, pH 5.2 and a_w 0.92, pH 5.4). For this reason all the values were averaged and the results are shown in Table 1 for both color functions, S_{uv} and L*. The Q^{10} values [$\log Q^{10} = (2.189\ E_a)/(T(T + 10))$] were also calculated. Activation energies for the color functions were almost identical, which indicated their equivalence as indicators of browning development in concentrated whey. Petriella et al. (1985) calculated activation energies for color development in stored glucose-lysine samples having a_w in the range 0.90–0.95 and pH 5 and 6; values reported were somewhat higher (35 to 26 Kcal/mole) than those corresponding to whey. This can be explained by the different nature of Maillard's reactants in both cases. The E_a found in the present work are, however, in good agreement with those reported by Labuza and Saltmarch (1981a) for whey powders stored at a_w = 0.65 (29–30 KCal/mole).

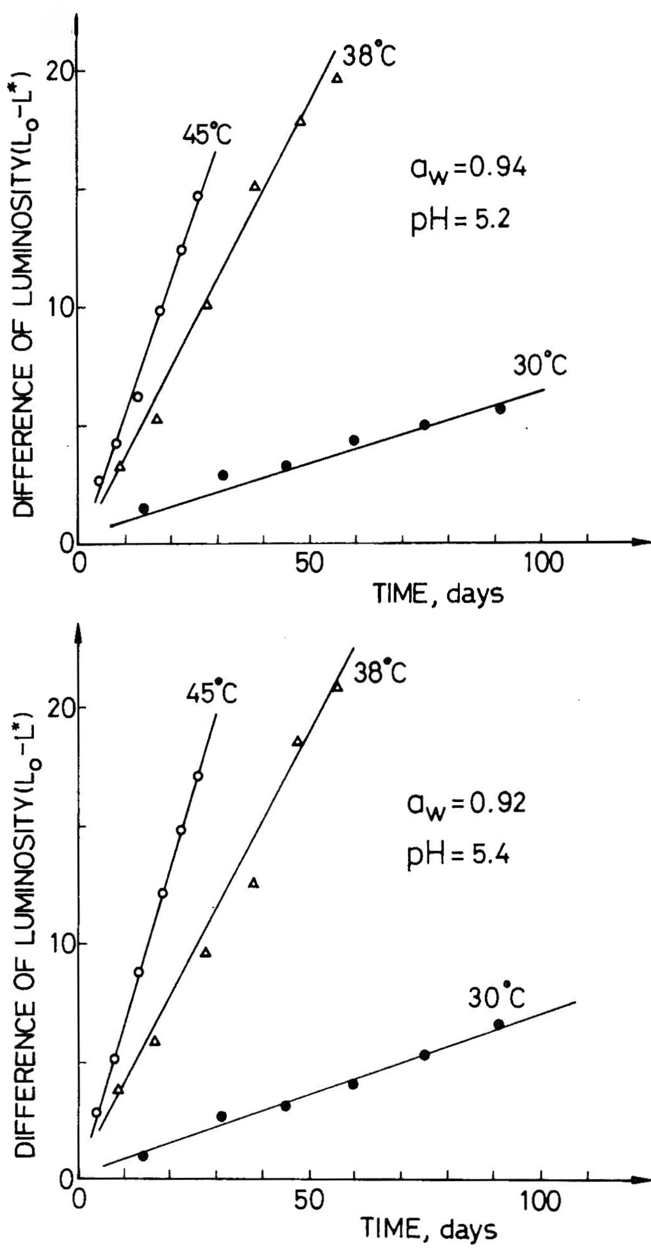

Fig. 2—Effect of heating time at different temperatures on the changes in luminosity for shelf stable concentrated whey samples.

Available lysine loss

The loss of ε-DNP-lysine in stored shelf stable concentrated whey samples was studied only at 30°C. The results are shown in Table 3. The loss of ε-DNP-lysine amounted to about 20–30% of the initial value after three months of storage at 30°C. Retention was slightly better for the sample having a_w 0.94, pH 5.2. No attempt was made to correlate the data with a kinetic model because of the relatively low degree of ε-DNP-lysine loss. For the purposes of comparison, Table 3 also shows the retention of chemically available lysine in whey powder of a_w 0.33 stored at 25°C and 35°C, as reported by Labuza and Saltmarch (1981a). The retention of available lysine in the

Table 1—Rate constants[a] and activation energies for color development in stored shelf stable concentrated whey

a_w	pH	30°C $k_{(S-S_o)_{uv}}$	30°C $k_{L_o-L^*}$	38°C $k_{(S-S_o)_{uv}}$	38°C $k_{L_o-L^*}$	45°C $k_{(S-S_o)_{uv}}$	45°C $k_{L_o-L^*}$
0.92	5.4	0.12	7.2	0.61	37.1	1.1	66.4
0.94	5.2	0.11	6.0	0.56	37.0	0.95	55.9

Activation energy (E_a)[b]

Color function	E_a[c] (kcal/mole)	Q_{10} (20–30 °C)	Q_{10} (20–40 °C)
$(S - S_o)_{uv}$	26.1 ± 2.5	4.4	4.0
$(L_o - L^*)$	26.1 ± 2.9	4.4	4.0

[a] $k_{(S-S_o)_{uv}}$ or $_{L_o-L^*}$ (hours^{-1} × 10^2)
[b] Average values for samples having a_w 0.94, pH 5.2 and a_w 0.92, pH 5.4.
[c] E_a ± 95% confidence limits.

Table 2—Retention of ε-DNP lysine in shelf stable concentrated whey samples stored at 30°C

Storage time (days)	Available lysine (% fraction remaining) Conc. whey[a] 30°C	Conc. whey[b] 30°C	Whey powder[c] 25°C	Whey powder[c] 35°C
0	100	100	100	100
31	92.1	99.3	96.5	80.7
60	91.4	93.5	93.3	66.1
91	70.9	83.0	90.1	53.3

[a] a_w 0.92; pH 5.4
[b] a_w 0.94; pH 5.2
[c] Calculated from data of Labuza and Saltmarch (1981 a) for whey powder of a_w = 0.33.

shelf stable concentrated whey samples of a_w 0.92–0.94 was similar to the retention values observed in whey powder of a_w 0.33.It is well known that a_w has an effect on the Maillard reaction; most studies indicate a maximum for the rate of Maillard reaction at a_w 0.6–0.8 (Labuza and Saltmarch, 1981b). For example, Loncin et al. (1968) reported a maximum for loss of lysine in dried milk in the 0.6–0.7 a_w range. Further increases of a_w decrease the reaction rate as a result of dilution. Thus, the high a_w in the shelf stable concentrated whey should act to slow down Maillard's reaction.

The relatively good stability of available lysine in the concentrated whey samples should also be attributed to the reduced pH. Petriella et al. (1985) showed that pH has a strong influence on the rate of Maillard's reaction in model systems of a_w in the range of 0.90–0.95.

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Mass Transfer During Brining of Cuartirolo Argentino Cheese

JULIO A. LUNA and JUAN A. BRESSAN

ABSTRACT

The mass transfer occurring during brining of the soft cheese "Cuartirolo Argentino," considered as a finite slab, is mathematically modelled as an effective diffusion process. Two alternative models were developed considering a well stirred brine and a brine at rest. Compared against experimental data they showed similar results. The theoretical and experimental NaCl concentration profiles showed good agreement, thus demonstrating the applicability of the simplest model.

INTRODUCTION

AS STATED PREVIOUSLY (Luna and Bressan, 1985) the purposes of the brining of Cuartirolo Argentino Cheese are to achieve cooling, uptake of salt, and moisture loss. Based on the characteristics of the process, and determining that the heat and mass transfer could be treated separately, the referred paper contained the analysis of the heat transfer process.

Brining was extensively studied by Geurts et al. (1974) on Gouda cheeses. Guinee and Fox (1983) used the same approach as Geurts et al. to study Romano cheese. Studies on soft type cheese have not yet been reported.

The objective of this work was to design a model to describe the uptake of salt into the cheese (moisture loss can be neglected) modelled as a pure diffusion process.

THEORY

THE SYSTEM under consideration (Luna and Bressan, 1985) consists of a brine and the cheese is assumed to be a finite rigid medium. During the brining process the brine is kept at a low constant temperature allowing the cooling of the cheese. Simultaneously, salt diffuses into the cheese and water is lost. It is assumed that there is no chemical reaction in the system, and that there is no convective mass transfer into the pores but only diffusion. Under the above assumptions it was demonstrated that the cooling of the cheese and the uptake of salt could be considered separately. Diffusion of water out of the cheese is neglected because, during the overall brining process, the difference in water content between the center and the side of the cheese is less than 3.5%. Accordingly, the governing equation for the NaCl diffusion into the cheese considered as a finite slab is:

$$\frac{\partial C(x,y,z,t)}{\partial t} = D_{eff}\,\nabla^2 C(x,y,z,t) \qquad (1)$$

where

$$-R_1 < x < R_1,\ -R_2 < y < R_2,\ -R_3 < z < R_3$$

For the case under consideration, experimental evidence shows that the initial concentration profile is flat. Then:

−I.C.: At $t = 0$ for any position.

$$C(x,y,z,0) = C_o = \text{constant} \qquad (2)$$

The brining process can be performed in a brine at rest and in a well-stirred brine. Accordingly two different sets of boundary conditions must be applied.

For the first case, a boundary condition (B.C.) of the third kind is applied as:

B.C.

$$\begin{aligned}
&\pm\frac{\partial C(\pm R_1,y,z,t)}{\partial x} + \frac{h_m}{\lambda_m}[C_c - C(\pm R_1,y,z,t)] = 0\\
&\pm\frac{\partial C(x,\pm R_2,z,t)}{\partial y} + \frac{h_m}{\lambda_m}[C_c - C(x,\pm R_2,z,t)] = 0\\
&\pm\frac{\partial C(x,y,\pm R_3,t)}{\partial z} + \frac{h_m}{\lambda_m}[C_c - C(x,y,\pm R_3,t)] = 0
\end{aligned} \qquad (3)$$

For low values of the Fourier number the approximate solution of the problem can be expressed as:

$$\frac{C-C_o}{C_c-C_o} \approx \left\{\text{erfc}\frac{1-(x/R_1)}{2\sqrt{F_{o_1}}} - \exp\left[B_{i_1}(1-x/R_1) + B_{i_1}^2 F_{o_1}\right] \cdot\text{erfc}\left[\frac{1-(x/R_1)}{2\sqrt{F_{o_1}}} + B_{i_1}\sqrt{F_{o_1}}\right] + \text{erfc}\frac{1+(x/R_1)}{2\sqrt{F_{o_1}}} - \exp\left[B_{i_1}(1+x/R_1)+B_{i_1}^2 F_{o_1}\right] \cdot\text{erfc}\left[\frac{1+(x/R_1)}{2\sqrt{F_{o_1}}} + B_{i_1}\sqrt{F_{o_1}}\right]\right\}\cdot\left\{\text{idem y-coordinate}\right\}\cdot\left\{\text{idem z-coordinate}\right\} \qquad (4)$$

where:

$$F_{o_i} = F_o\frac{R^2}{R_i^2};\ F_o = \frac{D_{eff}\cdot t}{R^2};\ \frac{1}{R^2} = \frac{1}{R_1^2} + \frac{1}{R_2^2} + \frac{1}{R_3^2};$$

$$B_{i_i} = B_i\frac{R_i}{R};\ B_i = \frac{h_m R}{\lambda_m};\ i = 1,2,3$$

The mass-exchange Fourier and Biot numbers are defined by Lykov and Mikhailov (1965).

For the second case (well stirred brine, $B_i - \infty$) a boundary condition of the first kind is applied as:

B.C.

$$\begin{aligned}
C(\pm R_1,y,z,t) &= C_c\\
C(x,\pm R_2,z,t) &= C_c\\
C(x,y,\pm R_3,t) &= C_c
\end{aligned} \qquad (5)$$

The solution of Eq. (1), with the initial and boundary conditions given in Eq. (2) and (5), can be expressed in a similar way as for the heat transfer case (Luna and Bressan, 1985) or by using a fast converging solution obtained as a product of the three one-dimensional solutions (Crank, 1975, Luikov, 1968):

$$\frac{C-C_o}{C_c-C_o} = \sum_{i=1}^{\infty}\sum_{j=1}^{\infty}\sum_{k=1}^{\infty}(-1)^{i+1}(-1)^{j+1}(-1)^{k+1}\cdot\left[\text{erfc}\frac{(2i-1)-x/R_1}{2\sqrt{F_o}} + \text{erfc}\frac{(2i-1)+x/R_1}{2\sqrt{F_o}}\right]\cdot\left[\text{erfc}\frac{(2j-1)-y/R_2}{2\sqrt{F_o}} + \text{erfc}\frac{(2j-1)+y/R_2}{2\sqrt{F_o}}\right]\cdot\left[\text{erfc}\frac{(2k-1)-z/R_3}{2\sqrt{F_o}} + \text{erfc}\frac{(2k-1)+z/R_3}{2\sqrt{F_o}}\right] \qquad (6)$$

Eq. (6) is very easy to use since only one term needs to be considered as reported by Luikov (1968) who analyzed the one-dimensional situation for F_o numbers between 0.001 and 0.1. The three-dimensional case is verified easily.

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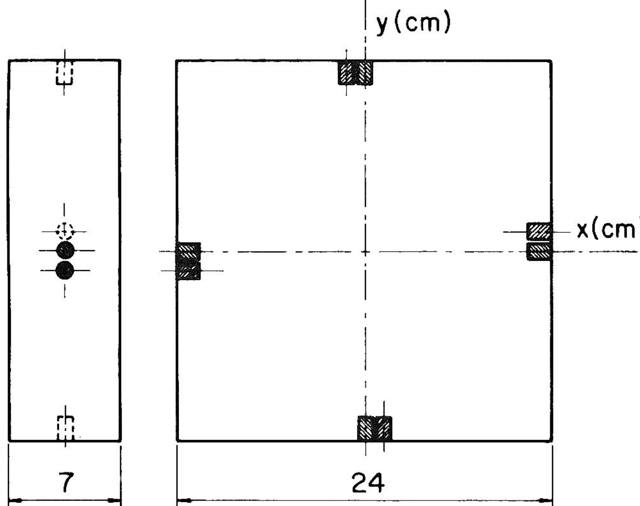

Fig. 1—Sampling points on each cheese. Eight samples (ϕ = 1 cm, h = 1.5 cm) were obtained for determining NaCl content.

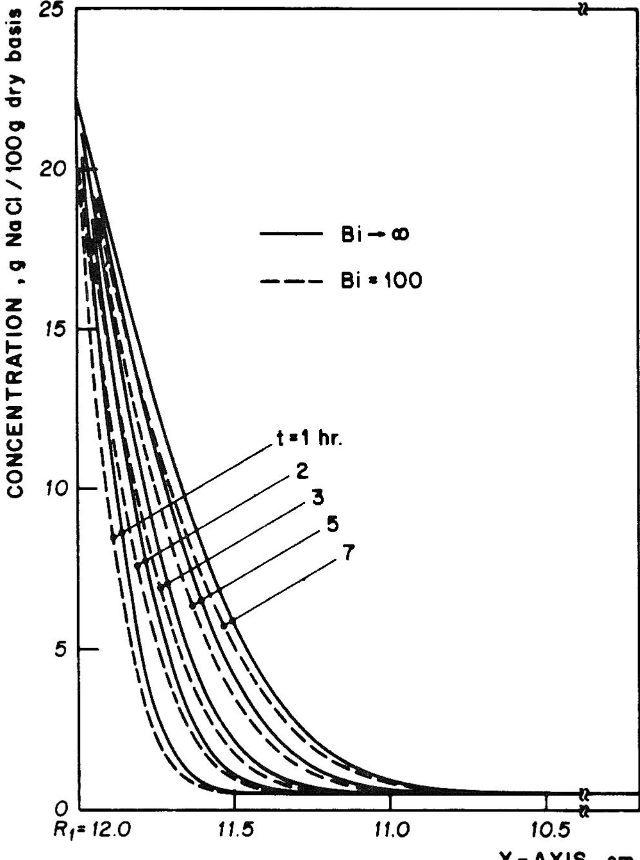

Fig. 2—Theoretical spatial concentration profiles during brining of Cuartirolo Argentino cheese at Biot of 100 and ∞. X-axis belongs to the domain $-R_1<x<R_1$; $-R_2<y<R_2$; $-R_3<z<R_3$.

MATERIALS & METHODS

Cuartirolo Argentino cheese

The Cuartirolo Argentino cheese used in this study is a soft type cheese made from whole milk as described previously (Luna and Bressan, 1985). The product used contained a moisture content of 52% and a fat content of 24.8%.

Sampling and brining experiments

For a 9-wk period, six molded cheeses a week, 24 cm × 24 cm × 7 cm in size, were taken from a commercial factory and transported to the pilot plant. After the cheeses reached the desired acidity one was used to measure the initial concentration profile (t = 0). The remaining five cheeses were brined for 7 hr in a 20.5°Be brine kept at 7.5°C. The brining process was performed in three different ways: (a) with agitation by brine recirculation with a 3000 lts/hr centrifugal pump; (b) with agitation by a turbine type agitator (ϕ = 15 cm, 4 blades, 1400 rpm); (c) with brine at rest. Cheeses were sampled at t = 1, 3, 5, 6, and 7 hr. From each cheese, eight samples (ϕ = 1 cm, h = 1.5 cm) were obtained as indicated in Fig. 1.

Table 1—Values of the positions and weights for evaluating Eq. 9[a]

i	A_i	ξ_i	z_i
1	0.08566	0.03376	0.05064
2	0.18038	0.16939	0.25408
3	0.23396	0.38069	0.57103
4	0.23396	0.61931	0.92896
5	0.18038	0.83060	1.24590
6	0.08566	0.96623	1.44934

[a] From Abramowitz and Stegun (1964), Table 25.8. A_i is weighting factor, ξ_i is dimensionless axial length and z_i is axial length.

NaCl determination

NaCl content was determined by measuring the Na content of the samples by Atomic Absorption Spectrophotometry (Juarez et al., 1983) using a Perkin Elmer 5000 Atomic Absorption Spectrophotometer.

Samples were prepared by adding demineralized distilled water to 1.8g cheese and then boiling the mixture (Have and Mulder, 1957) while adding 5 mL HNO_3 10N. The mixture was kept boiling until the digestion was completed.

RESULTS & DISCUSSION

THE EFFECTIVE MASS DIFFUSIVITY of NaCl was calculated as an extrapolation value from D_{eff} vs initial moisture content plots (% fat, dry basis as a parameter) (Geurts et al., 1974). For cheeses with 52% moisture and 51.6% fat (dry basis) a value of D_{eff} = 0.31 cm²/day was obtained.

By replacing this value in Eq. (4) and assuming different values of the B_i number, the evolution of the theoretical concentration profiles can be obtained. Similarly, by replacing the calculated D_{eff} value in Eq. (6) ($B_i \to \infty$) the theoretical concentration profiles for the well-stirred brine case can be obtained. Figure 2 shows the theoretical profiles on the x-axis during the overall brining process for $B_i = 100$ and $B_i \to \infty$. The theoretical profiles fall rapidly toward the initial concentration value at x values less than 1.5 cm. This fact needs to be taken into account when sampling the cheese to obtain the experimental data to be compared with the theoretical data. It was intended to obtain concentration data from samples 1 cm in diameter and 0.2 cm in height. Several cutting systems and devices were used but none of them rendered satisfactory results. A valid alternative was to obtain mean concentration values averaged over a cylindrical volume containing the concentration variations. From theoretical and experimental evidence a cylinder 1 cm in diameter and 1.5 cm in height was used to sample the cheeses and then to obtain the mean concentration values. These values needed to be compared to mean theoretical values defined as:

$$\bar{C} = \frac{\int_v C\, dv}{\int_v dv} \tag{7}$$

where C is a point concentration value given by Eq. (4) and (6). For a finite cylinder Eq. (7) transforms into:

$$\bar{C} = 2 \int_{\xi=0}^{1} \int_{r=0}^{1} C(\xi,r)\, r\, dr\, d\xi \tag{8}$$

where: $\xi \triangleq z/1.5$; $0 \leq z \leq 1.5$cm, $0 \leq \xi \leq 1$ being ξ and r the axial and radial normalized coordinate. The cylindrical samples are taken as indicated in Fig. 1. Due to the difference in

Table 2—Experimental and theoretical NaCl concentration data (at different Biot numbers) during brining of Cuartirolo Argentino cheese

Time (hr)	NaCl concentration[a] Experimental: Agitation: Pump	Turbine	No agitation	Theoretical: $\bar{C}_{B_i}$-∞ to $\bar{C}_{B_i}=400$	$\bar{C}_{B_i}=350$	$\bar{C}_{B_i}=300$	$\bar{C}_{B_i}=200$	$\bar{C}_{B_i}=100$
1	2.33(0.05)[b]	2.32(0.07)	2.23(0.05)	2.30	2.22	2.15	2.09	1.91
3	3.72(0.05)	3.69(0.04)	3.66(0.07)	3.67	3.67	3.67	3.47	3.25
5	4.50(0.07)	4.42(0.04)	4.48(0.04)	4.61	4.61	4.61	4.61	4.17
6	5.07(0.05)	5.03(0.04)	4.96(0.03)	5.01	5.01	5.01	5.01	4.57
7	5.34(0.04)	5.31(0.04)	5.33(0.06)	5.37	5.37	5.37	5.37	4.93

[a] g NaCl per 100 g dry basis.
[b] Numbers in parentheses represent pooled sample standard deviation (A Bartlett test was used to check homogeneity of variances).

Table 3—Percent deviation between experimental and theoretical NaCl concentration during brining process

		% Deviation[a]: B_i-∞ to $B_i=400$	$B_i=350$	$B_i=300$	$B_i=200$	$B_i=100$
Agitation	Pump	1.67	2.82	4.15	6.30	13.18
	Turbine	2.29	3.11	4.29	6.20	12.46
No agitation		2.23	1.60	2.39	4.37	11.15

[a] From Heldman (1974).

size between cheese and sample, and the characteristics of salt diffusion, the radial penetration can be neglected and then only taking into account the axial direction. Eq. (8) is then:

$$\bar{C} = \int_{\xi=0}^{1.0} C(\xi)\, d\xi \qquad (9)$$

The integral can be evaluated by using the Gauss numerical integration. The Gauss expression is:

$$\bar{C} = \sum_{i=1}^{m} A_i\, C(\xi_i) \qquad (10)$$

and the values of coordinates and coefficients were obtained from Abramowitz and Stegun (1964). The values of the A_i and ξ_i for the six first terms of the summation are listed in Table 1.

In this way, the theoretical concentration profiles given by Eq. (4) and (6) averaged according to the former procedure, can be satisfactorily compared with the corresponding experimental data; this is shown in Table 2. Table 3 shows the comparison of theoretical and experimental data by means of the percent deviation values (Heldman, 1974) according to:

$$\% \text{ Deviation} = \frac{\sum_{n=1}^{N} \left(\frac{VT - VE}{VE}\right)_n^2}{N-1} \times 100 \qquad (11)$$

where VT is the theoretical concentration value, VE is the experimental concentration value, and N is the number of points considered.

It shows that there are no significant differences between the two cases considered though Eq. (4) works better for brine at rest when Bi is 350. These results show that the model proposed in Eq. (6) describes adequately the intake of NaCl into a soft type cheese during the brining process. The proposed model can be used for any type of soft cheese with similar geometry provided the mass transport parameter is known.

CONCLUSIONS

The proposed theoretical model permits very good estimates of the uptake of salt into the cheese by using a D_{eff} value obtained by extrapolation of experimental data.

The problem solution given by Eq. (6) is very simple to use because only one term of the series needs to be considered.

The simplified theoretical model is important for the proper design and control of the brining process and the problem solution is the initial condition for the mass transfer problem which occurs during the ripening step.

NOMENCLATURE

B_i	Biot mass-exchange number
C	NaCl specific mass concentration (m NaCl/m_o)
$\bar{C}$	NaCl mean specific mass concentration
D_{eff}	Effective diffusivity (cm^2/day)
F_o	Fourier number
h	Height (cm)
h_m	Mass transfer coefficient, (g/cm^2 hr)
m_o	Mass of dry body, (g)
m NaCl	Mass of NaCl, (g)
R	Characteristic length, (cm)
t	Time, (hr)
V	Volume
x,y,z	Spatial coordinates, (cm)
λ_m	Mass conductivity (g/cm hr)
ϕ	Diameter, (cm)
ξ	Dimensionless axial length

Subscripts

o	Initial state
c	Surrounding medium

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A Research Note

Effect of Fat Source and Color of Lean on Acceptability of Beef/Pork Patties

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ABSTRACT

Twelve beef/pork meat blends were made from lean of mature beef and dark or mature pork carcasses. Meat blends containing about 20% beef fat were rated higher for appearance scores by a consumer panel than blends made with 20% pork fat. Visual acceptance of beef/pork patties was accomplished via use of all-beef fat and additionally by extending mature beef (MB) with mature pork (MP) or dark pork lean (DP). Agtron colorimetry scores indicate a minimum of 20% MB lean extended with 60% MP was needed to create the appropriate red color attractive to consumers. Use of 60% MP lean with about 20% beef fat resulted in patties acceptable to consumers for visual appearance and eating satisfaction.

INTRODUCTION

FRANCIS (1977) reported flavor was secondary in importance to color and visual appearance during the initial selection of foods. Consumers prefer the cherry red color of ground beef to the pale or grayish-pink color normally associated with ground pork (Parizek et al., 1981). Since dark pork lean is inferior to normal pork lean in consumer preference and is in less demand (Topel et al., 1976), this product may be a viable substitute to reduce the cost of ground beef and improve beef/pork patty color. Additionally, lean from packer sows is higher in myoglobin concentration than normal pork (Carmichael and Lawrie, 1967) and could also be used to extend and enrich color in a beef/pork blend.

One of the problems to overcome in a beef/pork product is flavor. However, since the species flavor and aroma of cooked meats are derived from the lipid portion of the muscle (Hornstein and Crowe, 1960), additions of only beef fat to beef/pork blends may improve flavor of the cooked product, reduce smearing and improve visual consumer acceptance.

Because dark pork lean and beef fat may enhance the sensory and visual characteristics of beef/pork patties, a study was designed to evaluate the effects of adding beef fat and dark or mature pork lean on the visual and eating traits of ground beef/pork blends.

MATERIAL & METHODS

Patty formulation

Twelve ground beef/pork blends (Table 1) were made from round or ham lean of mature beef and dark lean from mature sow carcasses. A 225 kg mature beef (MB, US Utility), a 170 kg sow (MP, US No 1) and five 75 kg market hog carcasses (DP, US No 1) with a lean color score of 4 or 5 (Anonymous, 1963) were deboned and physically separated into muscle and fat. Additional beef fat was separated from US Choice flanks. Lean and fat from each species was coarsely ground through a breaking plate with 1.3 cm openings, then mixed and formulated to contain the desired levels of lean and fat from each species with a lean-to-fat ratio of about 80:20. For beef/pork blends (3,5,7,8,9,10,11,12) specified to contain nearly all beef fat (BF), percentage fat from the defatted pork lean was determined by the Babcock procedure (AOAC, 1975) and BF was added to derive a total of 20% (about 5% pork fat and 15% BF).

Visual appearance panel

One hundred consumers evaluated blends for raw appearance using an 8-point, verbally anchored, hedonic scale with 8 = extremely desirable, 1 = extremely undesirable. Consumers were selected as they purchased their meat for their families. They were asked to evaluate each treatment in the fresh retail beef case under normal lighting and display conditions. Patties were evaluated in the meat case of a local supermarket.

Consumer panel-paired test

Each of 50 untrained consumers was presented 11, two-patty comparisons in three different sessions according to methods outlined by Amerine et al. (1965). These comparisons were of the control (80MB:20BF), and each of 11 other meat blends (Table 1). Thawed patties were placed on a rack 4.5 cm above the heating coils of a Farberware Electric Broiler unit (Model 450) and broiled on a 149°C grill surface for 5 min on each side to about medium-well doneness. Two (15g) sections (1 control, 1 treatment) were presented warm to panelists. All samples were presented to the panelists in a random order so preference to the order of presentation would not bias the results. Results were reported as a percentage of panelists preferring sample "A" or "B" or "same" based on eating satisfaction and cooked appearance. Thus, each of 50 consumers tasted samples in 11 comparisons for a total of 550 paired tests.

Colorimetry

Four random samples were taken from each blend and thawed 30 min at 25°C. Core samples were analyzed for relative reflectance with an Agtron Colorimeter using standard reflectance discs of 24 and 52.

Statistical analyses

Visual appearance panel and colorimetry data were analyzed by a one-way analysis of variance (Steel and Torrie, 1980) with blend as the main effect. When the main effect was significant across treatment, means were separated according to Duncan (1955). The predetermined level of probability was 5% for all analyses and will be used throughout this discussion. The paired-preference tests were estimated for significance by the methods outlined by Roessler et al. (1978).

RESULTS & DISCUSSION

FOR TWO DIRECT comparisons between blends made with nearly all beef fat (blends 1 and 3) vs blends made with nearly all pork fat (blends 4 and 6), patties made with beef fat were clearly rated higher for raw appearance scores (Table 1). Use of 20% pork fat for blends in this study resulted in fat smearing and a "fatty" appearance. Blends 9 and 11, formulated with 40% or 60% MP, were not different from an all-beef control (blend 1) for appearance scores or Agtron readings. Beef/pork patties formulated with DP (blends 8, 10 and 12) decreased linearly in appearance scores as the percentage DP increased

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Table 1—Means for raw appearance scores and Agtron readings for blends of beef/pork patties

Blend	Lean source, %[a]			Fat source, %[a]		Appearance score[b]	Agtron reading[c]
	MB	MP	DP	BF	PF		
1	80	(control)		20		6.6^{d}	22.0^{d}
2	80				20	3.8^{h}	53.5^{f}
3		80		20		5.2^{g}	54.5^{fg}
4		80			20	2.2^{i}	71.5^{gh}
5			80	20		4.0^{h}	69.8^{gh}
6			80		20	2.1^{i}	85.5^{h}
7	60	20		20		6.1^{ef}	29.5^{d}
8	60		20	20		6.6^{d}	34.8^{e}
9	40	40		20		6.3^{def}	34.3^{de}
10	40		40	20		6.0^{f}	49.5^{ef}
11	20	60		20		6.2^{def}	37.0^{def}
12	20		60	20		5.4^{g}	53.4^{fg}

[a] MB = mature beef, MP = mature pork, DP = dark pork, BF = beef fat and PF = pork fat.
[b] Mean values based on scores by 100 consumers with 6 = moderately desirable, 3 = moderately undesirable.
[c] Agtron colorimeter readings based on standard disks 24 and 52 with a 100 reading = light and 0 reading = dark.
[d,e,f,g,h,i] Means in a column with a common superscript are not different (P>0.05).

Table 2—Percentage consumer preferences for overall eating satisfaction/cooked visual appearance of an all-beef control patty vs 11 meat blends

Blend	Lean source[a]			Fat source[a]		Consumer preference, %[b]		
	MB	MP	DP	BF	PF	Blend preferred over control	Same	Control preferred over blend
1	80	(control)		20				
2	80				20	36.4	14.5	49.1
3		80		20		23.1	15.4	61.5
4		80			20	15.5	11.5	73.0
5			80	20		36.5	11.5	52.0
6			80		20	14.0	10.0	76.0
7	60	20		20		25.4	37.3	37.3
8	60		20	20		27.1	22.9	50.0
9	40	40		20		28.9	20.0	51.1
10	40		40	20		44.7	14.9	40.4
11	20	60		20		50.0	6.8	43.2
12	20		60	20		28.9	13.2	57.9

[a] MB = mature beef, MP = mature pork, DP = dark pork, BF = beef fat and PF = pork fat.
[b] Percentages based on 50 paired consumer preference tests for each of 11 control vs blend comparisons (total = 550 tests).

from 20% to 60% (Table 1). A blend made with 20% DP was equal to all-beef patties for raw appearance score. Thus, if DP lean from butcher hogs is used to formulate beef/pork patties additions above 20% will likely result in lighter colored patties with lower visual acceptability scores.

Blends 4, 5, and 6 contained all pork lean and were lighter in color and had lower appearance scores (Table 1) than other blends. Blends preferred by consumers (moderately desirable) also had colorimetry values less than 50. For five comparisons between MP (blends 3, 4, 7, 9, 11) or DP (blends 5, 6, 8, 10, 12), no clear advantage in visual appearance existed (Table 1). Therefore, it appears that a minimum of 20% mature beef lean, extended with up to 60% mature pork lean or 40% dark pork lean with maximum use of beef fat is needed to create a cherry red color and high raw appearance scores.

Ground beef (blend 1) was rated the same or preferred over ground pork (blends 4 and 6) by about 85% of consumers in eating quality (Table 2). Data shown in Table 2 indicate beef/pork blends 10 and 11 were equal or preferred to the all-beef control in eating satisfaction and cooked visual appearance. The number of consumers preferring blends formulated with MP (blends 7, 9, 11) increased as the percentage MP increased to 60% indicating that MP lean has a favorable affect on sensory properties of beef/pork patties. Among the three blends made with 20% to 60% dark pork, 40% DP blend was the same or preferred over the control by about 60% of the consumers which clearly exceeded preference ratings for blends with either 20% or 60% DP (Table 2).

These data indicate that beef/pork blends can equal or exceed the all-beef patty in consumer taste preference which concurs with the findings of Parizek et al. (1981). By using mature or dark pork lean as a lean source and maximizing use of beef fat, blends can be formulated which are preferred by consumers for visual appearance with acceptable eating satisfaction. Based on raw appearance scores (Table 1) and eating satisfaction and cooked visual comparisons (Table 2), the following three blends are recommended: 1st, blend 11 (20MB:60MP:20BF); 2nd, blend 10 (40MB:40DP:20BF); and 3rd, blend 7 (60MB:20MP:20BF). Since blend 11 is extended with 60% MP, and is statistically equal to the all-beef control for all visual and sensory traits studied, it could be formulated at the least cost per pound, and may prove beneficial to the food industry.

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A Research Note

Changes in the Melting Characteristics of Bovine Tendon Collagen Induced by a Bacterial Collagenase

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ABSTRACT

Differential scanning calorimetry was used to study the changes produced by a commercial *Clostridium histolyticum* collagenase preparation on the melting behavior of bovine Achilles tendon collagen. The samples were heated at 5°C/min from 25 to 100°C. As a result of proteolysis, the collagenase-treated samples were partially gelatinized at 25°C and, during the calorimetric experiments, exhibited significant decreases in their melting transition parameters when compared to intact collagen. The denaturation temperatures and enthalpies obtained were 61.6°C and 44.4 J/g sample for intact collagen and 43.6°C and 26.1 J/g sample for collagenase-treated collagen. These differences may be accounted for by changes produced in the structural organization of the collagen fibrils by the collagenase treatment.

INTRODUCTION

COLLAGEN is the most abundant animal protein and an important constituent of connective tissues (Aberle and Mills, 1983). These tissues are composed of a fibrous meshwork of collagen and elastin embedded in, and interacting with, an amorphous matrix of proteoglycans and glycoproteins. The molecular structure of collagen and its interactions with the other components of the connective tissue are significant factors influencing the textural characteristics of meat and meat products (Bailey, 1984). The basic collagen structure, known as tropocollagen, consists of three polypeptide chains, each twisted in a left handed helix, coiled around each other to form a right handed triple super helix (Aberle and Mills, 1983). At both the C- and N-terminus of the molecule there are short nonhelical segments called telopeptides which are the primary site for intermolecular cross-linking. Collage fibrils are formed from staggered and laterally cross-linked tropocollagen molecules; fibrils bound together form collagen fibers that make up the macroscopic arrangement detected as gristle in raw meat (Swatland, 1984).

As temperature increases, the regular structure of collagen breaks and the chains separate and fold into random structures, forming a gel. The nature and extent of cross-linking are believed by some researchers to be partially responsible for the thermal stability of collagen (Allain et al., 1978). Other factors affecting this thermal stability are the physical state (e.g., restrained or unrestrained) of the collagen (Snowden and Weideman, 1978; Macfarlane and Smith, 1978) and the water content of the tropocollagen arrangement (Luescher et al., 1974). The collagen structure is also susceptible to enzymatic degradation. Those enzymes capable of hydrolyzing peptide bonds in the triple helical region of undenatured collagen molecules are known as collagenases (Keil, 1979). This restrictive definition, however, excludes proteinases which selectively cleave native collagen molecules in the non-helical regions or degrade denatured collagen molecules. The most well-known bacterial collagenase is obtained from *Colistridium histolyticum*. This enzyme degrades native collagen sequentially inwards from the ends (particularly from the N-end), cleaving predominantly the bond Y-Gly in sequences of the type -Pro-Y-Gly-Pro- (where Y is most frequently a neutral amino acid), producing a complex mixture of low molecular weight fragments (Keil et al., 1975; Keil, 1979). The objective of the present work was to study the changes produced by a commercial collagenase preparation from *C. histolyticum* on the thermal stability of bovine Achilles tendon collagen.

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MATERIALS & METHODS

INSOLUBLE COLLAGEN from bovine Achilles tendon (Type V, No. C-4387) and *C. histolyticum* collagenase (Type IV, No. C-5138) were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were reagent grade or better. Collagen samples (50 mg) were suspended in 5 mL of 50 mM $CaCl_2$ in 1.0M Tris(hydroxymethyl)aminomethane buffer (pH 7.4) and rehydrated for 24 hr at 4°C. After the addition of 2.0 mg of collagenase, the mixture was kept at 25°C for 7 hr. The rehydrated native collagen and the collagenous residues remaining in the preparations after enzymatic digestion were washed thoroughly with 0.2M phosphate buffer (pH 7.4) prior to the calorimetric experiments.

Differential scanning calorimetry (DSC) experiments were performed in a DuPont Instruments 1090 Thermal Analyzer coupled to a 910 Differential Scanning Calorimeter cell (Du Pont Instruments, Wilmington, DE). Quadruplicate collagen samples, 5–10 mg of rehydrated native or hydrolyzed collagen covered with approximately 10 μL of phosphate buffer and sealed into aluminum pans, were heated from 25 to 100°C at a heating rate of 5°/min. A sealed pan containing an equivalent weight of phosphate buffer was used as reference. The thermal curves obtained were stored using a Dupont 1091 Disk Memory unit; an interactive data analysis program was used to calculate the thermodynamic parameters (enthalpy and temperature of denaturation) of the samples. The dry matter content of the collagens was determined by weighing the samples before and after freeze-drying.

RESULTS & DISCUSSION

REPRESENTATIVE thermal curves of intact and hydrolyzed collagen samples are shown in Fig. 1. The treatment of tendon collagen with the commercial bacterial collagenase produced a significant ($P < 0.01$) decrease in both the temperature and the enthalpy of denaturation (Table 1). These data resemble the denaturation temperatures of collagen fibrils and molecularly dispersed collagen (65 and 40°C, respectively) and may be the result of increased random water-protein interactions caused by a disruption of the fibrillar collagen arrangement. Water plays an important role in the stability of the collagen structure, probably through the formation of water bridges between the polypeptide chains (Privalov, 1982). The appearance of the collagenase-treated samples before heat treatment was that of thin collagen-like cores surrounded by an amorphous gelatinous substance.

The thermal stability of collagen is known to be dependent on the amount of water embedded within its structure; the denaturation temperature follows an inverse relationship with water content (Luescher et al., 1974). The covalent cross-links occurring between the telopeptides of one molecule and the triple helical parts of others help maintain the extensive but orderly water-mediated hydrogen bonding that keeps the col-

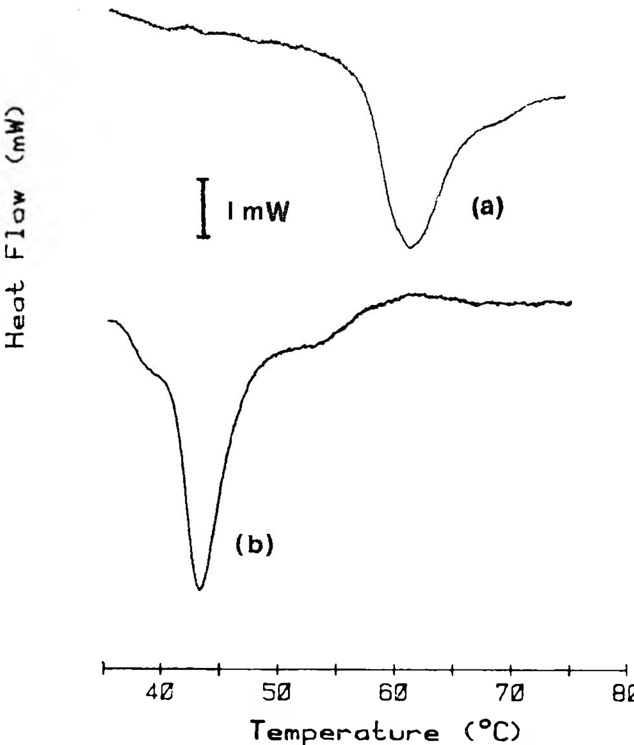

Fig. 1—Thermal denaturation curves of intact and collagenase-treated bovine tendon collagen in 0.2M phosphate buffer (pH 7.4): (a) Intact collagen; (b) Collagenase-treated collagen.

Table 1—Thermodynamic characteristics of intact and collagenase-treated collagen determined by differential scanning calorimetry

	T_D (°C)[a]	H (J/g dry sample)[b]
Intact collagen	61.55 ± 0.87[c]	44.36 ± 0.23
Collagenase-treated collagen	43.57 ± 0.88	26.12 ± 0.64

[a] Denaturation temperature. Heating rate = 5 °C.
[b] Enthalpy of denaturation (Joules/gram dry sample).
[c] Standard deviation of 4 samples.

lagen fibril tightly packed together and give it the strength required for its biological function (Miller, 1982). Upon proteolysis, the collagen network will be broken at the collagenase-cleaving points and the cross-links will no longer be sufficient to hold the triple helical structure packed together. As a consequence, water will penetrate the fibrous matrix making the connective tissue more susceptible to thermal denaturation. Molecularly dispersed mammalian collagens have melting temperatures in the 40°C range (Aberle and Mills, 1983; Bailey, 1984). These melting points are believed to reflect only the collapse of the collagen triple helix. The aggregation of tropocollagen into fibrils results in melting temperatures in the 65°C range. The results obtained in this work suggested that the collagenase treatment, by cleaving the collagen molecules, promoted the disruption of the noncovalent intermolecular bonds that reinforce the collagen fibril structure, enhancing random water-protein interactions and decreasing the temperature and the enthalpy of denaturation. As pointed out by Aberle and Mills (1983), these noncovalent intermolecular interactions must be more important for the stability of collagen against heat than the covalent cross-links, since only small variations in denaturation temperature (<5°C) are observed between collagens having marked cross-linking differences. The greater than 40% decrease in the enthalpy found with enzyme treated samples, thought to be produced by the disruption of intermolecular forces responsible for fibril packing, indicated that these forces were indeed important to the thermal stability of this material. The total heat of denaturation of the intact collagen sample must be the summation of the heat needed to disrupt the fibril arrangement plus the heat required to unfold the tropocollagen molecule. In the case of the collagenase treated sample, the heat of transition would be the result of the tropocollagen melting alone.

Collagen shrinks when meat is heated at temperatures above 70°C, generating a tension that compresses the meat fibers together; in addition, the residual strength of the denatured collagen determines the extent to which the muscle fibers adhere to each other (Bailey, 1984). These two factors seem to account for the increase in toughness observed when meat is heated from 70 to 80°C (Findlay et al., 1986); the myofibrillar proteins contribute to the development of meat toughness upon denaturation in the 40 to 55°C range (Bailey, 1984).

Enzymatic degradation of collagen may play a role in the tenderization of meat during aging (Stanley and Brown, 1973; Richardson, 1977; Bailey, 1984). Lysosomal enzymes have been observed to affect the thermal stability of collagen, decreasing the temperature at which epimysial collagen shrinks during heating and providing further evidence of the effect of proteolysis on the heat stability of collagen (Kopp and Valin, 1981). Eino and Stanley (1973) showed that treatment with collagenase produces a decrease in muscle breaking strength and suggested that this enzyme could be used to improve the textural characteristics of tough meat. The present work has shown that, apart from affecting the tensile properties of beef muscle, collagenase reduced the amount of heat needed to denature collagen and the temperature at which this denaturation took place. The reduction in the amount of heat needed to denature collagen and in the temperature at which this denaturation takes place may prove to be advantageous for the improvement of the eating quality of meat exhibiting collagen-related toughness. The characteristics required for a collagen-specific degrading enzyme before it could have a wide application in the postmortem tenderness improvement of tough meat cuts (having a high content of mature collagen cross-links) would be an optimum pH in the pH range of postmortem muscle (5.5–6.0), maximum collagenolytic activity at refrigeration temperatures, susceptibility to heat denaturation upon cooking and availability from a safe microbial source.

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A Research Note

Reduction or Replacement of Sodium Chloride in a Tumbled Ham Product

C. B. FRYE, L. W. HAND, C.R. CALKINS, and R. W. MANDIGO

ABSTRACT

Public concern over excess sodium in the diet has prompted investigation of sodium replacement in tumbled ham. Salt treatments consisted of a control (100% NaCl) and 50% or 100% ionic strength replacement with either KCl or $MgCl_2$. Protein extraction was monitored during the 8 hr, discontinuous tumbling cycle, and reached a peak within 6 hr for all salt treatments. Control hams had the best overall sensory scores, while KCl/NaCl treatment gave the best physical bind and acceptable sensory scores (P<0.05). Treatment with $MgCl_2$ gave the lowest bind and sensory scores (P<0.05). Partial replacement of NaCl ionic strength with 50% or less of KCl in tumbled ham can be accomplished while maintaining acceptable sensory and physical attributes.

INTRODUCTION

CONCERN about the effects of excessive sodium has prompted the consumer to consciously reduced dietary sodium intake. Currently, per capita consumption of sodium in the United States is 20-25 times over the minimum adult requirement (IFT, 1980). Efforts are needed to reduce sodium levels in processed meats, as these products are often perceived as being high in sodium (Terrell and Olson, 1981).

Sodium chloride (NaCl) plays a functional role in extracting proteins to create product bind (Schmidt, 1979). Research indicates that tumbling enhances this process (Krause et al., 1978; Ockerman et al., 1978; Ockerman and Dowercial, 1980). The potential for tumbling to facilitate reduction or replacement of NaCl needs investigation. The objective of this study was to determine to effects of KCl and $MgCl_2$ salts on texture and palatability of a tumbled ham product.

MATERIALS & METHODS

LEAN, BONELESS HAM PIECES (9.1 Kg/treatment for each of 2 replications) were pumped to 112% of green weight with a pickle solution which contained nitrite, sugar, erythorbate and 1 of 5 chloride salt treatments. The chloride salt treatments consisted of a 2% NaCl control (Na), and partial (50%) or complete (100%) replacement of NaCl with an ionic strength equivalent of potassium chloride (K/Na; K, respectively) or magnesium chloride (Mg/Na; Mg, respectively). After grinding through a 24 mm × 48 mm kidney plate, ham pieces were tumbled (11.5 rpm, 34.6 cm diameter drum; Vortron Sales, Ltd., Beloit, WN) under vacuum (635 mm of Hg) for 3 hr, held under vacuum for a 2 hr equilibration period and vacuum tumbled an additional 3 hr. The product was stuffed into 16.5 cm permeable casings, pressed in smokehouse racks, cooked and smoked to an internal temperature of 65°C. The cooked, boneless ham was then stored in a −2°C cooler for physical and sensory analyses within 1 mo.

Bind strength was assessed in two ways. Break force was defined as the force needed to break a 2.54 cm thick ham slice. The slice was bridged over a 6.6 cm span and a steel wedge was slowly (50 mm/min) driven into the midpoint. The Instron Universal Testing Machine (IUTM) was equipped with a 500 kg load cell and set for 100 mm/min chart speed.

Adhesion was defined as the force needed to pull apart a 4 mm thick ham slice at a speed of 200 mm/min. The IUTM was equipped with a 2 Kg load cell and set for 200 mm/min chart speed. Peak force and area under the curve (a measure of total work) were obtained for each test.

Protein extraction was determined by a new method. Small sponges (2.5 cm^3) were added with the ham pieces at the start of the tumbling cycle. Two sponges were removed 1, 2, 3, 6, 7 and 8 hr after the cycle was initiated. Total protein content of each sponge was determined by the AOAC Kjeldahl method (AOAC, 1980).

A consumer sensory panel (rep 1, n = 59; rep 2, n = 69) was used to evaluate flavor, texture, juiciness, overall acceptability and aftertaste on unheated product. Panelists used a seven-point, hedonic rating scale (7 = like extremely, 1 = dislike extremely).

Data were analyzed by analysis of variance. Means were separated by Duncan's new multiple range test (Steel and Torrie, 1980) and a regression line was plotted for protein extraction over time.

RESULTS & DISCUSSION

BREAK FORCE, adhesion and sensory scores for each treatment are shown in Table 1. Break force and adhesion revealed superior bind for Na and K/Na treatments and inferior bind for K, Mg or Mg/Na treatments, indicating that partial replacement of NaCl with KCl in a tumbled ham product can be achieved without reducing bind.

Sensory scores reveal that the control (Na) treatment had more desirable sensory attributes than all other treatments (Table 1). Although the K/Na treatment was rated lower than the control for each attribute, means for each attribute were associated with the desirable end of the rating scale. In a variety of products, Hand et al. (1982a,b,c) and Seman et al. (1980) found that full or partial replacement of NaCl ionic strength with KCl reduced texture and increased the intensity of bitterness and/or off flavor. Perhaps 50% replacement of NaCl with KCl is approaching the limitations of acceptability. Tumbled ham samples from the K, Mg/Na and Mg treatments were generally rated undesirable by the consumer panel, indicating that these treatments are not feasible alternatives for reduction or replacement of NaCl. Similar results were found by Hand et al. (1982c).

Protein extraction is shown in Fig. 1. There was no significant interaction between salt treatment and tumbling time. Thus, data were pooled across salt treatments and a quadratic regression equation was fit (Fig. 1). Means and standard errors at each sampling time are also presented on the figure. These means suggest that the majority of protein was extracted within 3 hr of tumbling. However, the plot of predicted values indicates that the 2 hr equilibration time between 3 to 5 hr was a significant factor related to protein extraction. Further research is needed to determine the importance of equilibration time and to examine the use of cellulose sponges to measure protein extraction in a tumbling system.

Protein extraction was also influenced by salt treatment. The Na and Na/K treatments extracted less (P<0.05) protein (9.47 ± 0.38 and 8.76 ± 0.37 mg/g of tumbled sponge, respectively) than did the Mg, Na/Mg or the K treatments (10.38 ±

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Table 1—Break force, adhesion and sensory scores of cooked and smoked hams made from either a total or partial replacement of NaCl with either KCl or $MgCl_2$

	Break force[a]		Adhesion[b]		Attribute[c]				
Treatment[d]	Peak (kg)	Area (cm^2)	Peak (g)	Area (cm^2)	Flavor	Texture	Juiciness	Overall acceptability	After taste
Na	1.80[e] (0.36)	9.62[e] (1.84)	22.37[e] (1.31)	19.34[e] (7.48)	5.41[e] (1.05)	5.51[e] (1.05)	5.16[e] (1.27)	5.37[e] (1.05)	5.34[e] (1.23)
K/Na	1.99[e] (0.54)	10.74[e] (1.85)	22.90[e] (2.59)	16.26[e] (5.28)	4.30[f] (1.28)	4.80[f] (1.29)	4.29[f] (1.27)	4.14[f] (1.24)	3.79[f] (1.48)
K	1.38[f] (0.46)	6.41[f] (2.74)	16.11[f] (2.82)	9.89[f] (3.72)	2.81[h] (1.43)	3.82[g] (1.51)	3.21[g] (1.51)	2.73[h] (1.39)	2.68[g] (1.47)
Mg/Na	0.62[g] (0.13)	2.88[g] (0.98)	3.58[g] (0.76)	2.18[g] (1.20)	3.54[g] (1.37)	4.06[g] (1.36)	3.42[g] (1.33)	3.46[g] (1.30)	3.59[f] (1.38)
Mg	0.56[g] (0.44)	2.76[g] (1.68)	2.40[g] (1.16)	1.50[g] (1.12)	1.75[i] (0.99)	2.33[h] (1.38)	2.30[h] (1.34)	1.83[i] (1.09)	1.90[h] (1.20)

[a] Boneless ham slices for break force were 2.54 cm thick.
[b] Boneless ham slices for adhesion test were 4 mm thick.
[c] Panelist rating on a seven-point, hedonic scale; 7 = like extremely, 1 = dislike extremely.
[d] Salt formulation treatments consisted of either 100% NaCl (Na), 50% KCl/50% NaCl (K/Na), 100% KCl (K), 50% $MgCl_2$/50% NaCl (Mg/Na), or 100% $MgCl_2$ (Mg).
[e,f,g,h,i] Means in the same column with different superscripts are significantly different ($P<0.05$). Parenthetical values indicate standard deviations.

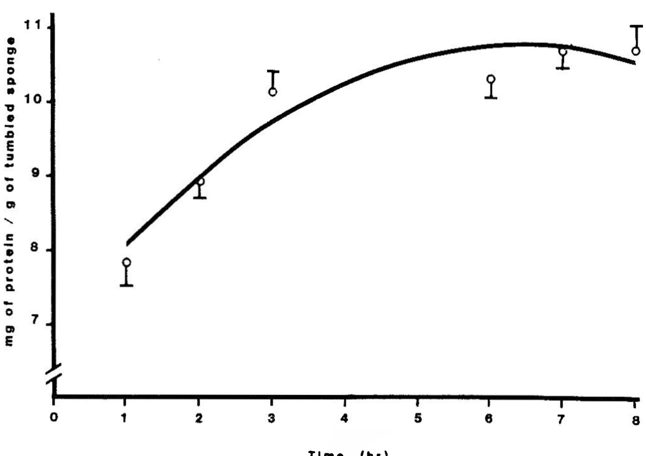

Fig. 1—Means, standard errors and predicted values ($\hat{Y} = 7.01 + 1.19 \cdot hr - 0.09 \cdot hr^2$; $R^2 = 0.3744$) for protein extraction during the tumbling cycle.

0.28, 10.35 ± 0.30 and 10.35 ± 0.39 mg/g of tumbled sponge, respectively). This contrasts with the textural data (Table 1), where treatments containing Mg were extremely low in bind and adhesion.

Data from this study suggest that $MgCl_2$ has detrimental effects on physical and sensory properties of tumbled ham, and that 100% replacement of NaCl with KCl has undesirable effects on sensory properties. Replacement of not more than 50% of the ionic strength of NaCl with KCl can produce a tumbled ham with acceptable sensory and binding properties.

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A Research Note

Percentage Ether Extractable Fat and Moisture Content of Beef Longissimus Muscle as Related to USDA Marbling Score

J. W. SAVELL, H. R. CROSS, and G. C. SMITH

ABSTRACT

Percentage fat and moisture values for beef longissimus steaks (trimmed completely of all subcutaneous fat, epimysium, and peripheral muscles) from 518 steer and heifer carcasses of A and B maturity over a wide range in marbling were as follows: Moderately Abundant = 10.42 and 68.14, Slightly Abundant = 8.56 and 69.56, Moderate = 7.34 and 70.35, Modest = 5.97 and 71.35, Small = 4.99 and 72.36, Slight = 3.43 and 73.61, Traces = 2.48 and 74.29, and Practically Devoid = 1.77 and 75.37.

INTRODUCTION

USDA (1980) marbling scores are an integral part of the USDA quality grading system for beef. Marbling, as subjectively evaluated in the longissimus muscle at the 12-13th rib interface, combined with maturity form the quality grades of U.S. Prime, U.S. Choice, U.S. Good, etc. One of the questions that arises quite often about the USDA marbling scores is how much fat — on a chemical basis — is associated with each marbling score. This information is important as the role of fat from animals in the human diet comes under closer scrutiny by the medical community and as the beef industry strives to better understand the composition of their products. This research note reports the percentage ether extractable fat and the percentage moisture of longissimus samples selected from a large cooperative study on beef grades conducted for the USDA. This information is very pertinent because it is recent, it covers a wide range of carcasses and the data on the grades of carcasses were obtained by a team of highly trained evaluators.

MATERIALS & METHODS

Steaks (1.27 cm in thickness) from the 13th rib region of one of the strip loins from each of 518 beef carcasses representing both steers and heifers, A and B maturity and ranging in marbling score from Moderately Abundant to Practically Devoid were used in this study. Complete details of the selection, preparation of steaks and results of palatability evaluations are reported in the studies of Smith et al. (1982, 1984).

After removal from each strip loin, all steaks were carefully trimmed of all subcutaneous fat, epimysium and peripheral muscles so that only the completely trimmed longissimus muscle was left. Each steak was frozen with the use of liquid nitrogen and powdered in a Waring Blendor. The blended muscle was placed in Whirl-Pak bags and stored in a −34°C freezer until the laboratory determinations could be made.

Samples from each carcass were analyzed for percentage ether extractable fat and percentage moisture following AOAC (1980) procedures. The longissimus samples (about 2g each) were loaded into prefolded oven-dried filter paper, weighed and placed in drying ovens (100°C) for at least 24 hr. Upon removal from the ovens, all samples were reweighed (for determination of moisture loss) and were subjected to ether extraction using Soxhlet apparati and diethyl ether. After an 8 hr extraction period, samples were removed, allowed to air to remove most of the ether, and placed in the drying ovens for at least 8 hr before reweighing (for determination of fat loss). All determinations were made in duplicate and only those samples that were within 10% of each other after computation to determine percentage fat and moisture were accepted (all others were reanalyzed until acceptable figures were obtained).

Means and standard deviations for percentage ether extractable fat and percentage moisture for each marbling score were calculated. In addition, a regression equation was developed in order to predict percentage ether extractable fat when marbling score is known. All analyses were conducted using the SAS (1982) package.

Table 1—Mean ether extractable fat and moisture contents of beef longissimus steaks stratified according to marbling level

		Ether extractable fat (%)		Moisture (%)	
Marbling level	n	Mean	SD	Mean	SD
Moderately Abundant	52	10.42	2.16	68.14	1.87
Slightly Abundant	61	8.56	1.60	69.56	1.39
Moderate	84	7.34	1.50	70.35	1.39
Modest	90	5.97	1.15	71.35	1.18
Small	80	4.99	1.10	72.36	1.06
Slight	80	3.43	0.89	73.61	1.06
Traces	47	2.48	0.59	74.29	0.78
Practically Devoid	24	1.77	1.12	75.37	0.99

RESULTS & DISCUSSION

PRESENTED in Table 1 are the means and standard deviations for the percentage ether extractable fat and percentage moisture for the steaks used in this study. The means range from 10.42 and 68.14 for Moderately Abundant to 1.77 and 75.37 for Practically Devoid for percentage fat and moisture, respectively. The most striking observation was that all of the mean values for percentage fat were relatively low when compared to the general perception by the public of the fatness of beef and when compared to the fatness levels in the various kinds of ground beef ("lean ground beef" has up to 20% fat).

The regression equation developed to predict the percentage ether extractable fat when the marbling score is known is as follows: Percentage ether extractable fat = (marbling score × 0.0127) − 0.8043 (the r-square for the equation is 0.7794). For this equation, marbling score is converted to a numerical code where Moderately Abundant = 800-899, Slightly Abundant = 700-799, Moderate = 600-699, Modest = 500-599, Small = 400-499, Slight = 300-399, Traces = 200-299, and Practically Devoid = 100-199. Thus, a Small-50 would be equal to 450, a Moderate-20 would be equal to a 620, etc.

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A Research Note

Potential Microbial Utilization of Citrus Oil-Mill Effluent

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ABSTRACT

Candida utilis (ATCC 9950) produced an average 0.21g protein per 100 mL citrus oil mill effluent while reducing the sugar content by 92.2% and the BOD by 33.8%. Limonene was removed from the effluent and sugars concentrated to 6.8% by membrane filtration. *Saccharomyces cerevisiae* (ATCC 4111) produced 3.0% ethanol by volume while reducing the sugar content 90.3% when grown in the concentrated effluent. *Hansenula holstii* (ATCC 13689) and *Rhodosporidium toruloides* (ATCC 10788) were grown in the concentrated effluent diluted to 2.7% sugars. Crude lipid produced by *R. toruloides* was 26.3% of the cell dry weight, while *H. holstii* produced 0.3 g crude extracellular polysaccharide per 100 mL.

INTRODUCTION

DURING A CITRUS PEEL oil recovery process, an aqueous discharge is generated having a recoverable oil content reported to range between 0.1 and 0.5% (Bryan et al., 1973; Steger, 1979; Braddock and Miller, 1982). The presence of the terpene, 1-methyl-4(methyl-ethenyl)cyclohexene (limonene) in the effluent is of concern because of its antimicrobial activity (Murdock and Allen, 1960; Subba et al., 1967; Veldhuis et al., 1972; Hong, 1977) which can severely disrupt traditional biological waste disposal systems resulting in reduced system efficiencies (Subba et al., 1967; Hong, 1977; Ratcliff, 1977).

Current disposal methods utilize an evaporation process which is often not economically efficient. This study was conducted to observe the production of single-cell protein (SCP), ethanol, extracellular polysaccharides, and lipids by various yeasts from this effluent. Such a process could result in an effluent having reduced limonene, soluble solids, and Biological Oxygen Demand (BOD), thereby making it less of a disposal problem.

MATERIALS & METHODS

EFFLUENT FROM A CENTRIFUGE (desludger) was collected from the peel oil recovery system at a typical citrus processor. SCP: *Candida utilis* (ATCC 9950) was grown in 100 mL pasteurized (82°C) effluent at 30°C for 4 days on a rotary shaker (150 rpm, 2 cm). Prior to inoculation, sugar (anthrone method; Stewart, 1975), BOD (APHA, 1975), and protein (biuret method, AOAC, 1980) of the pasteurized effluent were determined. At the end of the growth period, cells were harvested by centrifugation (1500 × *g*/10 min) and the supernatant assayed for BOD and percent sugar. The pellet was resuspended with distilled water to the sample weight prior to centrifugation for dry weight (g insoluble solids/100 mL) and protein determinations.

Ethanol

Limonene was removed and the solids concentrated in the effluent using ultrafiltration membranes (HFM 100 Abcor, Inc., Wilmington, MA) and reverse osmosis filtration (HR-95, DDS RO Div., Nakskov, Denmark) as described by Braddock (1982). Triplicate 300 mL samples were inoculated with *Saccharomyces cerevisiae* (ATCC 4111) and incubated 5 days at 30°C. After fermenting, cells were harvested by centrifugation (1500 × *g*/15 min) and ethanol (AOAC, 1980) and sugars measured. Dry weight and protein analyses were conducted as previously described.

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Table 1—Growth at 30°C of selected yeasts in pasteurized effluent

Organism	Colony forming units/mL		
	0 hr	24 hr	48 hr
Candida utilis (ATCC 9950)	< 10^3	1.1×10^8	5.9×10^7
Hansenula holstii (ATCC 13689)	2.2×10^6	1.3×10^4	1.7×10^4
Rhodosporidium toruloides (ATCC 10788)	2.4×10^6	3.2×10^4	1.8×10^4
Saccharomyces cerevisiae (ATCC 4111)	< 10^3	1.4×10^5 (est.)	2.0×10^4 (est.)

Extracellular polysaccaride and lipid

The substrates were 100 mL aliquots of pasteurized, membrane-concentrated effluent diluted to 2.7% sugar (Cadmus et al., 1962) and supplemented with 0.05% yeast extract and 0.1% KH_2PO_4. Triplicate 500 mL cultures of *Rhodosporidium toruloides* (ATCC 10788) and *Hansenula holstii* (ATCC 13689) were grown 4 days at 28°C on a rotary shaker (150 rpm, 2cm). After 96 hr, a sample was removed for protein analysis before harvesting the cells by centrifugation (2600 × *g*/15 min). From the supernatant, percent sugar and crude polysaccharide (Cadmus et al., 1963) were assayed. Cells from the pellet were freeze-dried prior to crude lipid analysis (Hunter and Rose, 1977).

RESULTS & DISCUSSION

CELL VIABILITY IN THE CENTRIFUGE effluent was determined by attempting to grow yeasts in pasteurized effluent. Only *C. utilis* produced significant growth in the pasteurized effluent (Table 1). This was assumed to be due to the antimicrobial activity of limonene (Graumlich, 1983). Ultrafiltration and reverse osmosis membrane filtration reduced limonene from 0.28% to 0.003% recoverable oil and concentrated sugars to 6.8%. This treatment made the effluent a better substrate for ethanol, lipid, and polysaccharide production by *H. holstii*, *R. toruloides*, and *S. cerevisiae*.

Protein production by *C. utilis* (Table 2) was not significantly different between non-supplemented and supplemented effluent. The effluent's overall protein content (g protein/100 mL effluent) increased over 100% due to growth of this organism. A potential application of the spent cells would be as a protein supplement for dried citrus pulp fodder produced by the industry. Due to reduced solids and limonene contents, treatment of the resulting effluent would be easier by traditional disposal methods; however, loss of limonene as a by-product would be a negative economic factor.

S. cerevisiae produced 3.01% (v/v) ethanol while reducing the sugar content of the membrane concentrated effluent by 90.3%. Crude extracellular polysaccharide and lipid production was conducted on the concentrated effluent which had been diluted to the approximate sugar content of the raw effluent thereby producing a medium similar in composition to the effluent with virtually no limonene. The medium was supplemented with 0.05% yeast extract and 0.1% KH_2PO_4. *H.*

Table 2—Production of protein, ethanol, lipid, and extracellular polysaccharide by selected yeasts

	Control	*Candida utilis*	*Candida utilis*	*Hansenula holstii*	*Rhodosporidium toruloides*	*Saccharomyces cerevisiae*
Growth time (hr)	9	96	96	96	96	120
Substrate[a]	PEU	PEU	PES	DEC	DEC	EC
Growth temp (°C)	--	30	30	28	28	30
Cell dry Wt (g/100 mL)	--	0.58	0.55	0.48	0.35	0.32
Protein conc (g/100 mL)	0.10	0.21	0.21	0.17	0.15	0.17
Protein yield g/g IS[b]	0.26	0.36	0.38	0.36	0.44	0.53
% Ethanol (by vol.)	--	--	--	--	--	3.01
Crude lipid						
% cell dry wt	--	--	--	7.7	26.3	--
g/100g sugar used	--	--	--	2.3	9.6	--
Crude ex. Polysaccharide g/100 mL	--	--	--	0.31	0.11	--
% Reduction:						
BOD	--	39.7	27.9	31.0	22.2	--
Sugar	--	91.3	93.1	60.4	35.2	90.3

[a] PEU = Pasteurized (82°C) Effluent Unsupplemented; PES = Pasteurized Effluent Supplemented with 0.5% $(NH_4)_2SO_4$ + 0.1% KH_2PO_4; EC = Effluent Concentrate (6.8% sugar); DEC = Diluted Effluent Concentrate (2.7% sugar).
[b] IS = Insoluble Solids.

holstii has been previously studied for polysaccharide production and produced almost three times more polysaccharide than *R. toruloides*. *R. toruloides* has been studied for its lipid accumulation ability (Bunker, 1955; Ratledge, 1979). This organism produced 26.3% of its dry weight as lipid as compared to 7.7% produced by *H. holstii*. The fat coefficient (g fat produced per 100g sugar consumed) of 9.6 is lower than the range of 16–18 reported by Bunker (1955), but much higher than that of *H. holstii*.

Considering the production of 11.4–30.3L of effluent per box of citrus (Kesterson et al., 1979; Steger, 1979) and the processing of over 160 million boxes (Citrus Summary, 1984), the amount of desludger centrifuge aqueous discharge produced by the U.S. citrus industry could potentially have been between 2 and 5 billion L. Applying results of this study and considering potential, this amount of effluent could have produced: (a) 5–14 million kg limonene; (b) 4–10 million kg SCP; (c) 20–56 million L ethanol; (d) 6–16 million kg crude extracellular polysaccharide; or (e) 6–18 million kg microbial lipids.

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A Research Note

Retention of Passion Fruit Juice Compounds by Ultrafiltration

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ABSTRACT

Recovery of passion fruit juice compounds by ultrafiltration was studied using a tubular system equipped with a 25,000 dalton nominal M.W. cut-off membrane. The retentivities of many important flavor compounds, such as β-ionone, ethyl hexanoate, hexyl butyrate and hexyl hexanoate, were 80% or higher when operating at 20°C and 12 bar of pressure. Retentivities of sugars and organic acids were between 10 to 40%. The retentivities increased as the concentration ratio increased.

INTRODUCTION

CONCENTRATION of fruit juices by membrane processes has claimed much attention in recent years (Merson and Morgan, 1968; Matsuura et al., 1974; Dale et al., 1982; Sheu and Wiley, 1983; Wilson and Burns, 1983; Kirk et al., 1983). The success of applying ultrafiltration (UF) and reverse osmosis (RO) to fruit juice concentration is largely dependent upon the retention characteristics of membranes for flavor components in the juices. RO is usually used to recover the fruit flavors. UF membranes, in general, are too porous to retain any flavor compounds in the juice. The purpose of this study, however, was to investigate the flavor distribution of passion fruit juice and the ability of ultrafiltration for recovering the flavor components in the juice.

MATERIALS & METHODS

Raw material

The passion fruit juice was extracted from the hybrid variety of *Passiflora edulis* Sims. (female) and *Passiflora edulis* var. *flavicarpa* (male) using a brush finisher equipped with 1.0 and 0.5 mm screens (Keeseng Machine Co., Tainan, Taiwan). The resulting juice was then screened through 80 mesh and 200 mesh screeners, followed by treatment with 100 ppm pectinase (Ultrazym 100, Swiss Ferment Co., Ltd., Switzerland) at 30°C for 1 hr to hydrolyze the pectic substances. The enzyme-treated juice was centrifuged to remove starch with a disc-type centrifugal separator (Model SA-1-02-135, Westfalia, W. Germany) at 9000 rpm (4950g) and 1 bar outlet pressure. To avoid enzymatic destruction of flavor components, the juice was pasteurized at 75°C for 40 sec (Chiou, 1984) using a plate heat exchanger (Model UHX, Iwai Industrial Co., Japan). The resulting passion fruit juice was then packaged and stored at −30°C for later use.

Ultrafiltration process

The PCI Laboratory RO/UF unit (Paterson Candy International Ltd., Witchurch, England) was used for ultrafiltration operations. This unit is a tubular system, equipped with B1 module, having a membrane area 0.9 m^2. The BX6 UF membrane with nominal molecular weight cut-off of 25,000 daltons was used. This membrane can be used between pH 2–12 up to 70°C with 3–15 bar of typical operating pressure. In this study, UF processes were operated at 30 L/min feed rate at 20°C. The operating pressure was 12 bar.

The retention characteristics of the UF membrane for various components in the juice were expressed as instantaneous retentivity (R), calculated by: $R = 1 - (C_p/C_b)$; where C_p = concentration of the component in the permeate; C_b = concentration of the component in the feed.

Analytical

The total solids of the samples were determined according to AOAC (1980) method.

For the analysis of volatile components (Buckholz et al., 1980; Chen et al., 1982), a 25 mL sample containing 5 μL ethyl cinnamate as internal standard was treated with nitrogen gas (flow rate 80 mL/min) for 2 hr at approximately 25°C to sweep the volatiles onto a 12 cm × 3 mm capillary tube containing 0.15g 80/100 mesh of Tenax-GC adsorption polymer (Supelco Inc., Bellefonte, PA). The adsorbed volatiles were desorbed and collected on a 3 m × 3 mm glass column packed with 3% Carbowax 20M on 80/100 mesh Chromosorb G/HP (E. Merck Co., Germany) by a procedure described by Chen et al. (1982). Gas chromatographic analysis was performed isothermally at 50°C for 5 min followed by programming from 50° to 115°C at 1°C/min and 115° to 195°C at 2°C/min. Identification of the major volatiles was accomplished by spiking with standard compounds in the sample and comparing the results with those reported by other researchers (Chen et al., 1982; Chiou, 1984; Kuo et al., 1985).

The sugars and organic acids were analyzed by HPLC (Waters Assoc., Milford, MA) (Chiou, 1984). The sugars were separated isocratically on a Lichrosorb NH_2 column (E. Merck Co., Germany) using acetonitrile/H_2O (75/25, V/V) as eluting solvent and a differential refractometer as detector (Model R-401, Waters Assoc.). A standard solution containing 1% fructose, 1% glucose and 1% sucrose (Sigma Chemical Co., St. Louis, MO) was used for identification of sugars in the sample. The organic acids were separated on a Lichrosorb RP-18 column (E. Merck, Germany) using a potassium dihydrogen phosphate and phosphoric acid buffer solution at pH 2.4 as eluting solvent, and detected at 220 nm with a variable wavelength detector (Model M-450, Waters Assoc.). Major organic acids were identified by comparing with a standard solution containing 0.5% citric acid, 0.1% L-malic acid and 0.1% lactic acid (Sigma Chemical Co., St. Louis, MO).

RESULTS & DISCUSSION

Sedimentation phenomenon and flavor distribution

Separation of passion fruit juice into two layers was observed in the laboratory when the juice was allowed to stand in a refrigerator for 1 or 2 days. The upper layer was slightly yellowish and transparent. The appearance of the lower layer was similar to that of concentrated juice, densely yellowish and turbid. When the two phases were carefully separated, it was found that the heavy phase possessed strong passion fruit juice flavor. The clear phase, on the other hand, smelled relatively plain. The volatile compounds of these two phases were analyzed by GC; the heavy phase [Fig. 1(B)] contained more volatiles than the light phase [Fig. 1(C)]. In addition, three of the four major esters in single strength passion fruit juice [Fig. 1(A)] were nearly all concentrated in the heavy phase. The four major esters were ethyl butyrate, ethyl hexanoate, hexyl butyrate and hexyl hexanoate (Hiu and Scheuer, 1961; Murray et al., 1972).

Retentivities of Volatiles by UF

Figure 1(D) and (E) illustrate GC flavor profiles of the diluted UF retentate and the UF permeate. Many of the compounds identified in this study were also found to be present in passion fruit as reported by other researchers (Hiu et al.,

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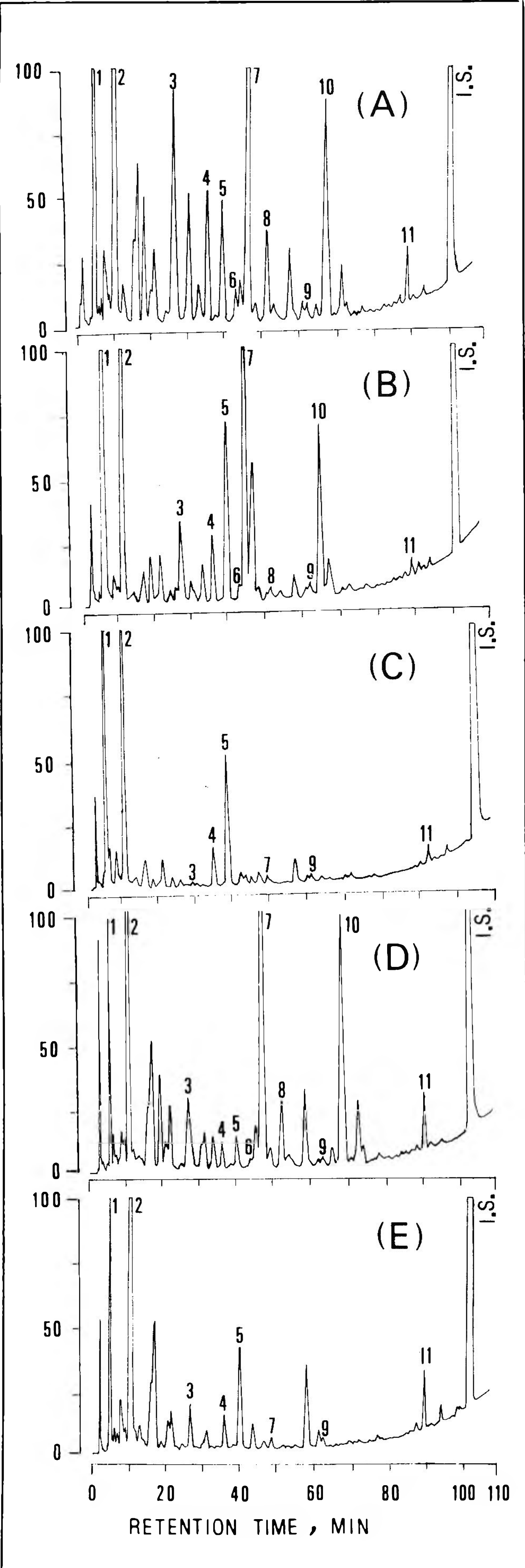

Fig. 1—Gas chromatograms of passion fruit juice volatile compounds. (A) single strength passion fruit juice; (B) heavy phase; (C) light phase; (D) UF retentate at weight concentration ratio (WCR) of 4. WCR = initial wt of feed/final wt of retentate. The sample was diluted with deionized-distilled water to its original weight before analysis; (E) UF permeate at WCR = 4. (1) ethyl acetate; (2) ethyl butyrate; (3) ethyl hexanoate; (4) 2-heptanol; (5) 1-hexanol; (6) cis-3-hexenol; (7) hexyl butyrate; (8) cis-3-hexyl butyrate; (9) linalool; (10) hexyl hexanoate; (11) β-ionone; I.S. = internal standard, ethyl cinnamate.

Table 1—Retentivities of passion fruit juice components at various concentration ratios with PCI BX6 UF membrane

	Retentivities[d] (%)		
Component	WCR[e] = 2	WCR = 3	WCR = 4
Total solids	24.9[a]	26.1[a]	28.5[b]
Acids			
Citric acid	12.8	13.1	13.7
L-Malic acid	10.0[a]	12.2[b]	14.3[c]
Lactic acid	17.9	19.9	21.2
Sugars			
Fructose	11.3	13.7	14.8
Glucose	13.3	14.6	13.6
Sucrose	34.8[a]	36.8[a]	31.5[b]
Flavor			
Total area[f]	66.4[a]	75.0[b]	83.2[c]
ethyl butyrate	19.9[a]	19.0[b]	18.0[c]
ethyl hexanoate	87.0	85.9	87.2
hexyl butyrate	99.2	99.5	99.5
hexyl hexanoate	100.0	100.0	100.0
β-ionone	65.6[a]	76.4[b]	81.3[c]
cis-3-hexenol	100.0	100.0	100.0
cis-3-hexyl butyrate	98.8	100.0	100.0
1-hexanol	43.8	42.8	46.6
linalool	69.1	72.2	83.3

[d] Retentivity (R) was calculated as, $R = 1 - (C_p/C_b)$; where C_p = concentration of the component in the permeate; C_b = concentration of the component in the feed.
[e] WCR (Weight Concentration Ratio) was calculated as: WCR = initial weight of feed/final weight of retentate.
[f] The retentivities were calculated based on the total peak areas of gas chromatograms of permeates and retentates.
[a,b,c] Significantly different at 5% level.

1961; Murray et al., 1972; Casimir et al., 1981). The retentivities of some major flavor compounds at various concentration ratios are given in Table 1. Many of the volatile aroma compounds were retained well by the UF membrane. Theoretically, the molecules of passion fruit volatile compounds are too small to be retained by the UF membrane. The fact that many of them were retainable during UF operations might be due to their association with large molecules in the juice. Under microscopic examinations, many small particles suspended in the juice were observed. These particles might have been the carriers of retainable volatile components.

Retentivities of nonvolatile compounds

The retention characteristics for sugars and organic acids of the UF membrane are given in Table 1. Major sugars of passion fruit juice are fructose, glucose and sucrose (Chan and Kwok., 1975). The most abundant organic acid in the juice are citric acid, L-malic acid, and lactic acid (Chiou, 1984; Chan et al., 1972). As expected, the retentivities of sugars and acids, in general, were very low.

The ability of UF membrane to retain many important passion fruit volatile compounds may be very useful. Possible utilization of this procedure would be recovery of the flavor compounds by UF. The permeate could then be concentrated by other means, such as evaporation, with better efficiency. The UF retentate, which would be higher in flavor, could be added back to the concentrated permeate to obtain the juice concentrate. —*Continued on page 844*

A Research Note

HPLC Analysis of HMF in Orange Juice

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ABSTRACT

An HPLC method for determining HMF (5-hydroxymethylfurfural) in orange juice has been developed. The method for HMF involves extraction of the juice with ethyl acetate prior to reverse-phase HPLC. Fortification studies indicate an average recovery of 96.1% ± 4.1% and a detection limit of 5.0 ppb.

INTRODUCTION

BOTH 5-HYDROXYMETHYLFURFURAL (HMF) and 2-furaldehyde (furfural) are useful indicators of temperature abuse in orange juice. HMF has been correlated with browning in orange juice (Meydav and Berk, 1978) while furfural has been shown to increase as flavor deteriorates in commercially processed orange juice (Nagy and Randall, 1973; Nagy and Dinsmore, 1974; Maraulja et al., 1973).

Quantitative determinations of these compounds in the past have depended on colorimetric measurements. A colorimetric procedure for determining furfural in orange juice was proposed and subsequently improved by Dinsmore and Nagy (1972, 1974). In 1984, two similar HPLC methods were developed (Marcy and Rouseff, 1984; Mijares et al., 1984) to quantify furfural in orange juice. These methods were more precise than earlier colorimetric procedures, and specific for furfural.

Two colorimetric methods are available for HMF quantitation. The International Federation of Fruit Juice Producers (IFFJP Method No. 12, 1974) published a procedure based on the reaction of the aldehyde with barbituric acid and p-toluidine. Meydav and Berk (1978) proposed a method using the more stable color reaction of HMF with thiobarbituric acid. Both methods require strict control of reaction time and temperature to achieve stable, reproducible absorbance readings and neither of the methods is specific for HMF. Brause and Raterman (1982) suggested HPLC could be used to determine HMF as part of a method for verifying apple juice authenticity.

Inconsistent results from either colorimetric method led us to develop an HPLC procedure for determining HMF in orange juice. The HPLC method offers substantially improved accuracy, sensitivity, and specificity as compared to the colorimetric procedures.

MATERIALS & METHODS

CHROMATOGRAPHIC SOLVENTS, and the ethyl acetate extractant, were High Purity UV Grade (J.T. Baker, Phillipsburg, NJ). HMF was obtained from Aldrich Chemical Company, Inc., Milwaukee, WI. Gas chromatographic analysis (HP 5880, Hewlett Packard, Palo Alto, CA) using a CP Sil 8 CB (12 m × 0.1 mm, Chrompack USA, Inc., Bridgewater, NJ), indicated 98.2% purity. Linearity by HPLC was established using different standard concentrations (10, 50, 150, 300, 700, and 1400 ppb). A daily working standard (150 ppb) was prepared in distilled water containing 0.2% phosphoric acid.

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Table 1—Colorimetric and HPLC results of HMF analyses on juice samples (37°C)

Sample	Storage time (wk)	Colorimetric (ppb)	HPLC (ppb)
1	0	700	32
2	0	720	21
3	0	740	19
1	2	720	293
2	2	770	229
3	2	750	227
1	4	810	790
2	4	820	595
3	4	800	605

HPLC system

A Spectra-Physics 8100 HPLC with a 100 μL loop injector and 8400 UV/VIS detector (Spectra-Physics, San Jose, CA) operated at 285 nm was used. Peak area was calculated with a Spectra-Physics 4100 computing integrator. A reverse-phase column was used for the analysis (Spherisorb 5 C8, 250 × 4.6 mm i.d., HPLC Technology, Inc., Palos Verdes, CA). The mobile phase consisted of 5% acetonitrile in water containing 0.2% phosphoric acid.

Sample preparation

Single strength orange juice was centrifuged at 47,800 × *g* for 20 min. The partially clarified juice was filtered through Whatman No. 1 filter paper (Whatman Ltd., England) and 25 mL of the filtrate was titrated to pH 7.0 (± 0.2) with 5N sodium hydroxide. The sample was extracted (4 × 25 mL of ethyl acetate), combined extracts evaporated to dryness in a rotary evaporator at 30°C and the residue dissolved in 2 mL water. The samples were filtered through a Gelman GA-8 membrane (0.2 μm) filter (Gelman Sciences, Inc., Ann Arbor, MI) before injection into the HPLC.

Fortification study

Twenty-three mL aliquots of single strength Valencia juice were fortified with 2 mL aqueous standard ranging from 45–1000 ppb. An unspiked juice sample made up of 23 mL juice and 2 mL water was used as the blank. Recovery of HMF by extraction from the spiked juices and the blank was measured.

Colorimetric analysis

The procedure of Meydav and Berk (1978) was used to determine HMF content of orange juice.

Storage of orange juice

Three samples of freshly produced Valencia orange concentrate were diluted to 11.8° Brix and bottled in 8 oz glass bottles with screw caps. Sodium benzoate (0.1% w/v) was added to retard microbial growth. The bottles were stored at 37°C and individual bottles were periodically evaluated for HMF content by colorimetric and HPLC procedures.

RESULTS & DISCUSSION

HMF ELUTES in less than 10 min using the conditions employed in this work. Confirmation of peak identity and purity was established by comparison of retention times and stop-flow scanning. Good correlation of the standards and corresponding peak areas ($r^2 = 0.996$) over the range 10–1400 ppb was established. The average standard deviation among tripli-

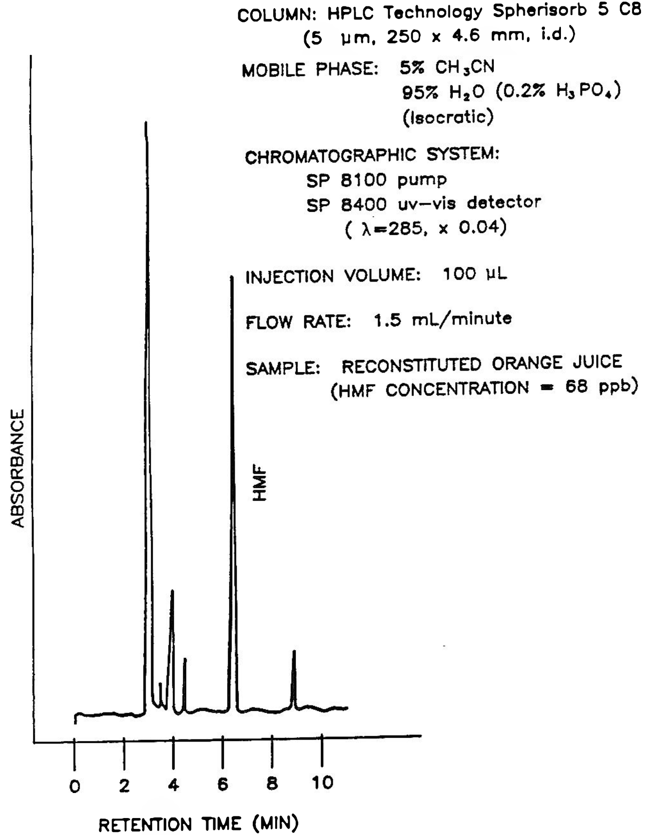

Fig. 1—HMF in orange juice.

cate injections was 0.64% (n = 63). Fortification studies indicated average recovery of 96.1% with a standard deviation of 4.1% (n = 42) over the range 45–1000 ppb. The detection limit for the analysis is 5.0 ppb.

Results obtained by the colorimetric and HPLC analyses of HMF (Table 1) suggest no correlation between the two methods ($r^2 = 0.146$). Little difference in HMF concentration was found between three samples of Valencia orange juice by colorimetric analysis. HPLC results indicated a significant difference in the HMF contents of these juices. After 4 wk of storage at 37°C, HPLC results indicated a 25–32 fold increase in HMF concentration whereas the increase indicated by the colorimetric procedure was very slight (1.1–1.2 fold increase).

In summary, HPLC analysis gives a rapid, accurate, and reproducible measurement of HMF in orange juice. The method should have applications to the study of browning and flavor changes occurring during processing and storage of orange juice.

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A Research Note

Sugar Content of Baked Sweet Potatoes from Different Cultivars and Lengths of Storage

DAVID H. PICHA

ABSTRACT

Sugar concentrations in baked roots of six different sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars were determined at harvest, after curing (32°C, 90% RH for 10 days), and during 46 wk of storage at 15.6°C. Maltose was the major sugar and sucrose the secondary sugar in all cultivars at harvest. Maltose decreased during curing and over long term storage. Sucrose, glucose, and fructose concentrations increased during curing and through at least 4 wk of storage in the orange-flesh cultivars. Sucrose concentration was always greater than either monosaccharide. Cultivars differed in sugar concentration and pattern of sugar change during storage.

INTRODUCTION

CARBOHYDRATES are the main constituents of the dry matter portion of baked sweet potatoes. During baking, much of the starch is converted into dextrins and maltose by α- and β-amylase (Walter et al., 1975). Other sugars found in baked roots are sucrose, glucose, and fructose (Picha, 1985). Carbohydrate composition influences a number of textural and organoleptic attributes (Jenkins and Gieger, 1957b; Reddy and Sistrunk, 1980; Walter et al., 1975). The principal reason for consumer purchase of sweet potatoes is the sweet taste (Law, 1977). Limited information is available on individual sugar content of currently grown cultivars held for different lengths of storage.

Flavor evaluations performed in this laboratory over the past several years indicate a wide range in flavor exists within the sweet potato germplasm. This stimulated interest in determining baked product sugar content in a range of high to low dry matter cultivars.

The purposes of this study were: (1) to determine the sugar content in baked roots of currently grown orange-flesh cultivars along with several high dry matter, white-flesh cultivars; and (2) to determine the magnitude of sugar change after curing and storage.

MATERIALS & METHODS

TWO WHITE-FLESH cultivars (Whitestar, Rojo Blanco) and four orange-flesh cultivars (Centennial, Jewel, Jasper, Travis) of sweet potatoes were grown in Baton Rouge, Louisiana, following commercially recommended procedures (Montelaro et al., 1966). Sugar and moisture contents of baked roots from each cultivar were determined the day of harvest, after curing (10 days at 32°C; 90% RH), and in cured roots after 4, 14, 30, and 46 wk of storage (15.6°C; 90% RH). Twenty-four randomly selected No. 1 grade roots per cultivar were divided into four replications at each analysis time. Each replication consisted of the combined flesh from six different roots.

Whole roots were baked in a Magic Chef conventional oven (American Stove Co., St. Louis, MO) for 75 min at 190°C. After cooling, roots were cut longitudinally in half and moisture content of the edible flesh was determined by drying duplicate 10.00g samples from each replication at 70°C for 48 hr in a forced air oven. An additional 10.00g portion of edible flesh was extracted in 80% ethanol and analyzed for sugar content by HPLC as previously described (Picha, 1985). Results for sugar content were expressed on a fresh weight basis.

Alcohol-insoluble solids (AIS) content of the raw roots was determined by homogenizing 10.00g of flesh tissue in 80% ethanol for 1 min at high speed using a Virtis 45 homogenizer. The resulting slurry was immediately boiled for 15 min, cooled and filtered through Whatman #4 paper. The weight of the insoluble residue retained on the filter paper was determined after 24 hr of vacuum drying at 35°C.

Statistically significant differences ($P < 0.05$) between storage durations within a cultivar in the amount of each component were determined by analysis of variance and means were separated according to Fisher's Least Significant Difference (LSD) test (Snedecor and Cochran, 1967).

RESULTS & DISCUSSION

BAKED ROOT moisture content among cultivars was inversely related to raw root AIS content (Table 1). After 4 wk of storage (14 wk in Whitestar), moisture content of baked roots generally increased with increasing storage time. AIS content decreased in all cultivars during curing and continued to decrease in the four orange-flesh cultivars with increasing lengths of storage. A slight increase in AIS content occurred in Whitestar and Rojo Blanco during the early part of storage, but then generally declined with increasing lengths of storage. Most of the raw root AIS fraction consists of starch (Hammett and Barrentine, 1961). Previous workers using other cultivars also reported decreases in starch during storage (Hammett and Barrentine, 1961; Ali and Jones, 1967; Sistrunk et al., 1954).

Maltose was the major sugar in Whitestar, Rojo Blanco, and Centennial, and was the major sugar in the other three orange-flesh cultivars until the latter periods of storage (Table 1). The order of rank in maltose concentration among cultivars paralleled that of AIS. The entire maltose content of baked roots resulted from the baking process, since no maltose was found in the raw root. Sweet potato starch gelatinizes at 68–73°C and α- and β-amylase are unable to attack ungelatinized starch (Walter et al., 1976). α-Amylase hydrolyzes starch by an endo process into amyloid polymers called dextrins (Ikemiya and Deobald, 1966; Walter et al., 1975). β-Amylase hydrolyzes starch and starch fragments into maltose (Balls et al., 1948; Walter et al., 1975). Altered β-amylase activity as a result of curing or the formation of an enzyme inhibitor which is then degraded during the first weeks after curing may explain why maltose concentration was less after curing than 4 wk of storage (except in Travis). The decline in maltose concentration after 4 wk of storage in all orange-flesh cultivars and after 14 wk in the white-flesh cultivars may be due to less available substrate (starch) for β-amylase.

Sucrose was generally the secondary sugar in all cultivars, but became the primary sugar in Jewel, Jasper, and Travis roots during the latter storage periods (Table 1). Sucrose content in all cultivars increased after curing and through at least 30 wk of storage in the four orange-flesh cultivars. Others also found nonreducing sugars increased after curing and short-term storage in several different orange-flesh cultivars (Jenkins and Gieger, 1957a; Ali and Jones, 1967; Walter and Hoover, 1984). Sucrose decreased in Whitestar and Rojo Blanco after curing

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Table 1—Moisture, alcohol-insoluble solids (AIS), and sugar content of six sweet potato cultivars at harvest, after curing, and during 15.6°C storage

Cultivar and storage duration	Moisture %[a]	AIS %[b]	Maltose g/100g[a]	Sucrose g/100g[a]	Glucose g/100g[a]	Fructose g/100g[a]	Total Sugars g/100g[a]
Whitestar							
At harvest	57.9	29.6	13.31	1.82	0.11	0.11	15.34
Cured[c]	60.2	26.6	10.15	4.21	0.27	0.25	14.88
4 wk	60.0	28.0	12.74	3.27	0.38	0.45	16.84
14 wk	58.0	28.9	13.92	3.15	0.36	0.40	17.83
30 wk	60.8	26.7	12.88	3.09	0.40	0.43	16.80
46 wk	62.6	26.2	11.87	3.14	0.47	0.45	15.93
LSD (0.05)[d]	1.9	1.4	0.87	0.62	0.12	0.11	0.87
Rojo Blanco							
At harvest	64.5	24.0	11.17	1.77	0.20	0.18	13.32
Cured	66.5	21.9	8.62	2.81	0.84	0.57	12.84
4 wk	65.6	22.7	10.74	2.32	1.22	0.86	15.14
14 wk	66.4	22.1	10.77	1.59	1.22	0.97	14.55
30 wk	67.5	20.6	9.67	2.12	1.26	0.85	13.90
46 wk	68.4	17.6	7.92	2.68	1.36	0.83	12.79
LSD (0.05)	1.8	1.3	0.95	0.44	0.27	0.19	0.57
Centennial							
At harvest	64.4	22.5	11.12	2.41	0.08	0.07	13.68
Cured	69.5	19.8	8.95	4.29	0.14	0.19	13.57
4 wk	66.7	19.6	10.15	5.40	0.19	0.29	16.03
14 wk	67.4	19.3	9.33	5.47	0.37	0.37	15.54
30 wk	70.2	16.9	7.64	5.84	0.56	0.51	14.55
46 wk	72.9	13.9	6.54	5.62	0.76	0.50	13.42
LSD (0.05)	2.1	2.3	0.96	0.59	0.18	0.15	0.95
Jewel							
At harvest	68.6	19.4	9.70	2.63	0.15	0.11	12.59
Cured	70.8	17.8	7.06	3.90	0.62	0.48	12.06
4 wk	69.5	17.6	8.32	4.06	1.17	1.05	14.60
14 wk	71.0	16.1	7.55	4.18	1.19	1.30	14.22
30 wk	71.3	14.6	6.58	5.01	1.32	1.32	14.23
46 wk	75.0	12.3	4.81	5.37	1.89	1.63	13.70
LSD (0.05)	1.7	1.5	0.91	0.73	0.43	0.35	1.04
Jasper							
At harvest	73.2	17.3	8.35	2.36	0.11	0.10	10.92
Cured	71.4	16.3	6.94	3.91	0.24	0.20	11.29
4 wk	72.5	16.1	8.39	4.47	0.46	0.42	13.74
14 wk	72.9	15.4	7.75	5.14	0.42	0.41	13.72
30 wk	76.5	12.8	5.51	5.68	0.47	0.41	12.07
46 wk	77.5	11.0	4.29	5.57	0.58	0.42	10.86
LSD (0.05)	1.6	1.1	1.06	0.51	0.13	0.09	0.88
Travis							
At harvest	76.4	12.4	5.99	1.40	1.06	1.02	9.47
Cured	76.2	12.0	5.72	2.08	2.25	1.54	11.59
4 wk	77.8	10.8	4.90	3.22	2.47	1.72	12.31
14 wk	79.0	9.3	4.02	3.26	2.63	1.89	11.80
30 wk	80.3	8.0	3.35	3.61	2.80	1.92	11.68
46 wk	80.6	6.9	2.56	3.81	2.49	1.66	10.52
LSD (0.05)	1.3	1 2	0.94	0.48	0.51	0.33	0.70

[a] Fresh weight basis of baked roots.
[b] Fresh weight basis of raw roots.
[c] Cured = After 10 days at 32°C, 90% RH.
[d] Least significant difference.

and continued to decrease in Rojo Blanco until at least 14 wk of storage. The different pattern of sucrose change between white and orange-flesh cultivars indicated that either different sucrose metabolizing pathways existed or that enzyme activities varied with storage.

Glucose and fructose, the least concentrated sugars in baked sweet potatoes, increased in all cultivars after curing and during the first 4 wk of storage (Table 1). Further increases during longer term storage generally were found only in Centennial and Jewel. Glucose concentration was higher than fructose in Rojo Blanco and Travis only. Travis had the highest monosaccharide concentration among cultivars. Whitestar, Centennial, and Jasper all contained similar glucose and fructose concentrations, but less than the other three cultivars.

Total sugar concentration was highest after 4 wk of storage in all cultivars except Whitestar (Table 1). A decrease in baked root total sugar content generally occurred with increasing raw root storage time. Whitestar had the highest and Travis the lowest total sugar content. Centennial generally contained the most total sugars of all orange-flesh cultivars. Other workers also reported more total sugars in baked sweet potatoes after curing than at harvest (Hammett and Barrentine, 1961), and after several months storage compared to after curing (Ali and Jones, 1967; Sistrunk et al., 1954).

The amount and kind of sugars differed among cultivars and storage time of raw roots. In general, the largest sugar changes occurred during curing and the first 4 weeks of storage. Sensory evaluations are needed to determine the relationship between individual sugar concentration and consumer preference.

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A Research Note

Effects of Process Stage and Storage on Retention of Beta-Carotene in Tomato Juice

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ABSTRACT

Twelve tomato cultivars were evaluated during commercial juice production and storage for retention of beta-carotene. Samples of fresh tomatoes, after extraction, pasteurization, canning, and after 7 months storage at 22° C were analyzed according to the AOAC methods (1980) for beta-carotene. There was a twofold difference in beta-carotene content between the highest and lowest cultivars in raw processed and stored product. A 20% loss of the beta-carotene content was observed during extraction and another 20% loss found after 7 months of storage.

INTRODUCTION

THE IMPORTANCE of beta-carotene in a normal healthy diet has been well documented and accepted (NAS, 1980). Recent trends in research have also suggested that beta-carotene may have a positive role in the prevention of some types of cancer (Mayer and Goldberg, 1984).

Fruits and vegetables are the major food source of beta-carotene in the human diet. Gould (1983) reported the nutritive value of these foods to be influenced by cultivar, climate, cultural practices, harvesting, maturity, processing and storage. Tomatoes, a member of both the fruit and vegetable families, have been shown by past research to be a significant source of beta-carotene (McCollum, 1955; Yamaguchi et al., 1960). With the development of the mechanical harvester in the 1960's, the characteristics for desired tomato cultivars have changed. Beta-carotene evaluation has not been performed on these newer developed cultivars.

The effects of processing and storage on beta-carotene retention is as much of a concern to the food technologist as is the raw product's nutritional value. Studies have shown different food products to retain vitamin A activity at varying rates. Carrots are very stable in provitamin A retention during storage (Langley et al., 1933). Yellow corn, however, has been shown to lose 50% of vitamin A activity during an 11 month storage period (Fraps and Triechler, 1933). Fellers (1940) found short storage periods to be of no harm to vitamin A activity in foods; however, longer storages showed slow but progressive losses of the vitamin. Studies by Kohman et al. (1931), Borenstein and Bunnell (1966), and Youssef (1982) reported the vitamin A activity in tomatoes to be essentially unaffected by commercial canning processes. If the vitamin A activity in tomatoes is stable, then newer cultivars higher in beta-carotene content would be very beneficial to the nutritional value of tomato juice.

The objectives of this research were: (a) to evaluate the beta-carotene levels in newer tomato cultivars used in tomato juice manufacture; (b) to determine the effect of manufacturing on beta-carotene levels in tomato juice; (c) to determine the effect of storage of tomato juice on beta-carotene levels.

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Materials and Methods

Processing and sampling: Twelve processing tomato cultivars were selected for evaluation from the 1984 tomato crop grown at the Ohio Agricultural Research and Development Center, Vegetable Crops Branch near Fremont, Ohio. The tomatoes were grown in replicate plots according to acceptable commercial practices. Each cultivar was machine harvested and transported by bulk handling to the Food Processing Pilot Plant at The Ohio State University, Columbus. The raw tomatoes were then processed within 24 hours according to commercial practices for tomato juice. A screw type Langsenkamp (with screen size 0.023") was used for the extraction and a Walker Wallace paraflow heat exchanger (Model H7) was used to pasteurize the juice.

Samples for beta-carotene evaluation were obtained in duplicate from each cultivar at the following steps during processing: raw, washed; after extraction through a 0.023" screen; after pasteurization at 121°C for 42 seconds; immediately after canning; and after 7 months of storage at 22° C.

Beta-carotene analysis: Beta-carotene was assayed using the AOAC method for fresh plant materials and silages (AOAC Sec. 43.014, 1980). The method consisted of three steps. (1) Extraction of the total pigments with acetone:hexane solvent (40:60). (2) Separation of carotenoids by column chromatography, using activated magnesia. (3) Determination of beta-carotene spectrophotometrically at 436 nm.

Statistical analysis: The data was analyzed using the SAS package (Statistical Analysis Systems Institute, Cary, NC). Duncan's multiple range tests were run to separate significantly different means. ANOVA tables were constructed to indicate significant variances and interactions.

Results and Discussion

The beta-carotene determinations for each cultivar and sample are shown in Table 1. Significant differences in beta-carotene levels were found between the cultivars at each processing step ($p<0.05$). Cultivars OE 3046, OE 3021 and Ohio 8239 were the highest in beta-carotene content at each step. After processing and storage, a significant 2-fold difference was observed in beta-carotene content between the highest and lowest tomato cultivars.

Analysis of variance showed no significant interactions between each of the cultivars during processing or during storage ($p>0.05$). The effects of processing and storage are therefore reported on pooled cultivar data (Table 2). Significant losses of beta-carotene occurred only during the extraction step and during storage. The mean percent retention of beta-carotene after extraction was 80.1%. After 7 months of storage, 59.7% of the beta-carotene content of tomato juice was retained.

The National Academy of Science (1980) recommends an intake of 1000 retinol equivalents (RE) for males 21 years or older. The percent contribution of RDA per 6 oz. serving of juice after canning and storage is presented according to cultivar in Table 1. The highest contribution for vitamin A activity in the fresh canned samples was 26.39% from cultivar OE 3046 and less than 2% loss of the recommended requirement was observed during storage. A difference in vitamin A RDA contributions from each of the cultivars was 2-fold for both the fresh canned and stored juices.

Table 1—Comparison of beta-carotene content in tomato juice by sample and cultivar[a]

Cultivar	Raw, washed	Extracted[b]	Pasteurized[c]	Canned	Canned,[d] stored	% of adult male RDA provided by 6oz juice: Canned	% of adult male RDA provided by 6oz juice: Canned, stored
	Retinol Equivalents[e]/100g						
OE 3046	209.0a	149.0a	167.5a	145.0a	138.0a	26.39	25.12
OE 3021	205.0ab	149.0a	145.0ab	123.0ab	119.5bc	22.39	21.75
Ohio 8239	160.0abc	149.0a	156.5a	141.5a	123.0ab	25.75	22.39
OE 3694	160.0abc	123.0ab	108.0bc	97.0bcd	71.0d	18.39	18.93
Peto 95-43	156.0abc	152.0a	112.0bc	101.0bcd	104.0c	17.65	12.92
Ohio 7814	130.5abc	93.5bc	75.0c	82.5cde	63.5de	15.65	12.92
Ohio 7870	130.5abc	101.0bc	101.0bc	86.0cd	71.0d	19.02	14.29
OE 3642	116.0bc	93.5bc	104.5bc	86.0cd	48.5ef	12.92	14.29
Camp. 4135	112.0c	93.5bc	112.0bc	71.0de	78.5d	15.65	8.83
OE 3734	108.0c	93.5bc	104.5bc	104.5bc	78.5d	15.02	11.56
Ohio 7983	93.5c	75.0c	67.5c	52.0e	41.0f	22.39	11.56
Heinz 2653	93.5c	67.5c	75.0bc	123.0ab	63.5de	9.46	7.46

[a] Means within each column having unlike letters are significantly different (p<0.05) (n=2)
[b] Screen size 0.023"
[c] 42 seconds at 121°C
[d] 7 months at 22°C
[e] 6 micrograms beta-carotene = 1 Retinol equivalent

Table 2—Effects of processing and storage on beta-carotene content of tomato juice[a]

Sample	Mean Retinol Equivalents per 100g	Mean % Retention from fresh
Raw, washed	139.50a	---
Extracted juice (0.023" screen)	111.67b	80
Pasteurized (42 sec @ 121°C)	110.71b	79
Canned	101.04b	72
Canned, stored (7 mo. @ 22°C)	83.33c	60

[a] Means having unlike letters are significantly different (p<0.05) (n=24)

The results of this study show that processing does significantly affect beta-carotene levels in tomato juice, but not enough to reduce its value as a good source of vitamin A. Choice of cultivar was found to be the important factor in obtaining the highest levels of vitamin A activity in the tomato juice. Further research in the area of tomato cultivar selection and vitamin A activity would help to insure tomato products having the highest nutritional status.

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A Research Note

Water-Soluble Components in Coffee Beans: Photoacoustic Assessment

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ABSTRACT

Photoacoustic spectroscopy offers a possible nondestructive alternative for in situ assessment of water-soluble compounds in green or roasted coffee beans. Its relevance to fast, routine evaluation of coffee beans is discussed.

INTRODUCTION

IN THE PAST FEW YEARS, photoacoustic spectroscopy (Rosencwaig, 1981; McClelland, 1983) has evolved into a powerful analytical technique specially suited when standard transmission or reflection methods are difficult to use. In recent studies (Lima et al., 1983; Cesar et al., 1984) on green coffee, a conspicuous photoacoustic spectral feature has been reported in the 340 to 380 nm region which disappears in direct proportion to the severity of roasting.

The present work provides an application of photoacoustic spectroscopy to a nondestructive assessment of water-soluble components in coffee beans.

MATERIALS & METHODS

LIGHT from a modulated source (Oriel 400 Watts Xe lamp and PAR — chopper, 30 Hz Princeton Applied Research Inc.) was passed through a standard monochromator (Jarrell-Ash Model 82-020) operating in the 300 to 800 nm range onto a homemade high gain brass photoacoustic (PA) cell fitted with both a high transparency window and a high sensitivity microphone (Brüel and Kjaer 4166). A lock-in amplifier (PAR Model 191) was used for signal detection and the reference channel fed with a signal from the chopper. For normalization a piezoelectric detector (Molectron P1 Series) was used as a lamp blank.

Samples of freshly ground and roasted coffee beans were compacted into a disk-shaped sample chamber in the PA cell holder. Roasting was carried out at 230°C, i.e., above the known thermal decomposition thresholds for both trigonelline and chlorogenic acids. Samples were collected at several roasting times and PA spectra taken immediately after sample preparation.

To separate specific groups of compounds for their contributions to the observed spectral absorption in the 360 nm region, sequential extractions were made with a Soxlet apparatus.

RESULTS & DISCUSSION

FIGURE 1 shows the PA spectrum of ground green coffee taken from 340 to 610 nm. A distinctive broad peak centered at 360 nm is evident. PA spectra of sequential extractions, starting with pure coffee sample, indicated that only the water-soluble fraction displayed the 360 nm feature. It thus appears that this peak is associated with the water-soluble components of coffee. The chlorogenic acids are known to absorb strongly around the 300 nm region with a recorded log ϵ = 3,4 at the 320 nm absorption peak (Bradfield and Flood, 1952). However, both the transmission and PA spectra of analytical grade chlorogenic acids indicate that Fig. 1 peak was off centered as compared to the peak in the pure substance spectra. This could be indicative of a contribution from other substances. Though it cannot be firmly established here, the potential contribution

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Fig. 1—Photoacoustic spectrum of whole ground green coffee.

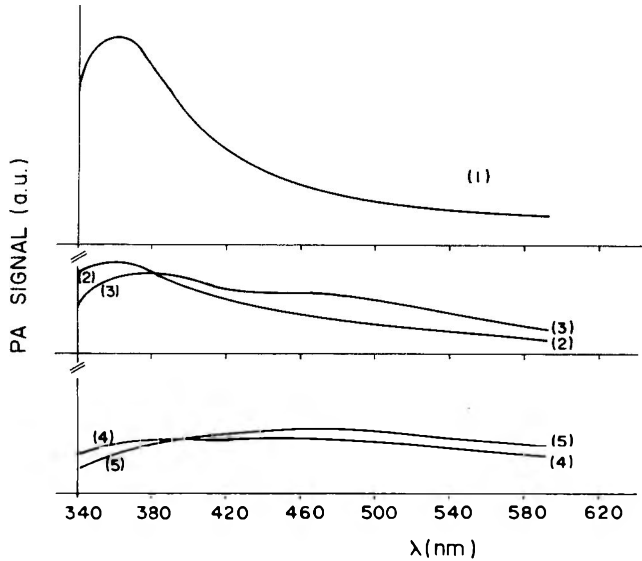

Fig. 2—Water-soluble compounds in coffee: effects of thermal degradation at 230°C upon the PA spectrum. Spectra 1 to 5 are for samples from coffee beans roasted for 0, 2, 3, 5, and 7 min, respectively.

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A Research Note

Water Uptake and Flour Gelatinization of Three Dry Bean Cultivars (*Phaseolus vulgaris* L.)

G. N. AGBO, M. A. UEBERSAX, G. L. HOSFIELD, and P. MARKAKIS

ABSTRACT

The water uptake and starch pasting of three dry bean (*P. vulgaris* L.) cultivars, Sanilac, San Fernando and Nep-2, were examined in an effort to understand certain palatability differences among them. Sanilac displayed the highest water uptake, followed by Nep-2 and that by San Fernando. Brabender amylograms on whole bean and decorticated bean flours did not show sharp pasting peaks, but paste viscosity of all cultivars increased on cooling, indicating retrogradation occurred. The San Fernando flour pastes were the least viscous among the three cultivars; the pastes of the other two cultivars differed slightly in viscosity. Water absorption and pasting viscosity do not fully explain differences in palatability among these cultivars.

INTRODUCTION

DRY BEANS (*Phaseolus vulgaris* L.) are a world-wide food staple requiring hydration and cooking before ingestion. The water absorption rate and capacity of beans have been shown to be affected by heredity, cultivation conditions, storage conditions, and amendments to the soaking medium. The starch and protein of the seed are greatly involved in the hydration process. Seed anatomy and cellular structure influence the hydration of the beans (Agbo, 1982). There has been considerable interest in using starch and protein fractions of bean flours in bread, cookie and other product formulations. Starch hydration and gelatinization characteristics influence the final quality of beans used in the canning industry.

In developing dry bean cultivars, plant breeders use selection strategies to insure that the food quality of the beans is maintained or improved (Bliss and Hall, 1977). The underlying genetic premise used by the breeders is that beans of similar genetic constitution (genotype) will have similar food quality characteristics. However, in the case of two dry bean strains maintained by the Michigan Agricultural Experiment Station that differ only by a single mendelian gene for seed coat color (black for San Fernando and white for Nep-2) (Moh, 1971), there were demonstrable differences in processed food quality (Agbo, 1982). The differences in palatability between San Fernando and Nep-2 could be due, partly at least, to water uptake and starch gelatinization disparities. The objective of this study was to provide data on the hydration and thermal pasting characteristics of genetically different bean cultivars with a view to facilitating breeding efforts for improving the culinary qualities of beans.

MATERIALS AND METHODS

DRY BEANS (*Phaseolus vulgaris* L.) of the cultivars Sanilac, San Fernando and Nep-2 were grown in nurseries at the Michigan State University Botany farm (East Lansing, MI) in 1978, 1979 and 1980. Seeds were threshed from mature plants, cleaned of broken seeds and debris, and sized using metal sieves. In experiments in which the seed coats were separated, the beans were soaked in warm water for 5 min and seed coats were removed by hand. The beans were ground with a Cyclone Sample Mill (UD Corp.).

Moisture was determined by oven-drying at 80 ± 2°C until the weight remained constant. Crude fat, ash and protein (Micro-Kjeldahl) were determined by AOAC methods (1975). Starch was assayed by a modified AACC (1969) method involving hydrolysis of the starch by glucoamylase (EC 3.2.1.3 from Sigma Chem. Co.) and determination of the hydrolysis product, glucose, by high-pressure-liquid-chromatography (Waters Assoc. μBondapack/carbohydrate column). Water uptake was determined by soaking 10g samples of beans in a 1:1 mixture of tap and distilled water at ambient temperature (ca 22°C) for 0, 15, 30, 45, 60, 75, and 90 min, followed by draining on a paper towel for 1 min and reweighing them. The pasting profiles of whole bean flours and coatless bean flours were obtained by using the Brabender Visco/Amylograph (AACC, 1969). Quantities of flour corresponding to a standard 50g — 14% moisture sample and 400 mL phosphate buffer, pH 5.3, were used in this test.

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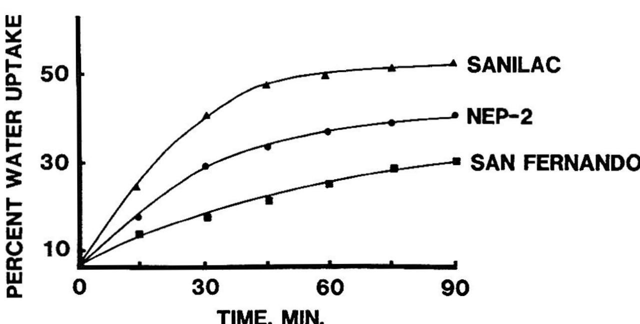

Fig. 1—Water uptake by beans of the cultivars Sanilac, Nep-2 and San Fernando at 22°C (averages of three crop years).

RESULTS & DISCUSSION

PROXIMATE ANALYSIS of the dry beans (3 samples × 3 years × 3 cultivars) showed no statistically significant ($P = 0.05$) differences in the content of the following constituents, given in weight averages: moisture 7.3%; protein 23.8%; starch 47.4%; fat 1.3%; and ash 4.4%. For the same cultivar, the year-to-year variability in proximate composition was rather large. Hosfield et al. (1984) found wide variabilities for several nutritional and culinary quality traits of beans.

A composite drawing of the water uptake patterns of the three bean cultivars for the three crop years is shown in Fig. 1. The Sanilac beans hydrated faster and to a higher level than the beans of the other two cultivars. Nep-2 is intermediate and San Fernando is the least hydratable among the three cultivars. Neither protein nor starch content differences can explain the variable water uptake among the cultivars. In subsequent work an explanation based on microanatomical features of the beans was sought (Agbo, 1982).

The pasting profiles of flours prepared from whole beans and from decorticated beans are shown in Fig. 2. The curves show no distinct viscosity peaks during heating, and they are similar to the pasting curves of other legume flours and starches (Colonna and Mercier, 1979; Lineback and Ke, 1975; Schoch

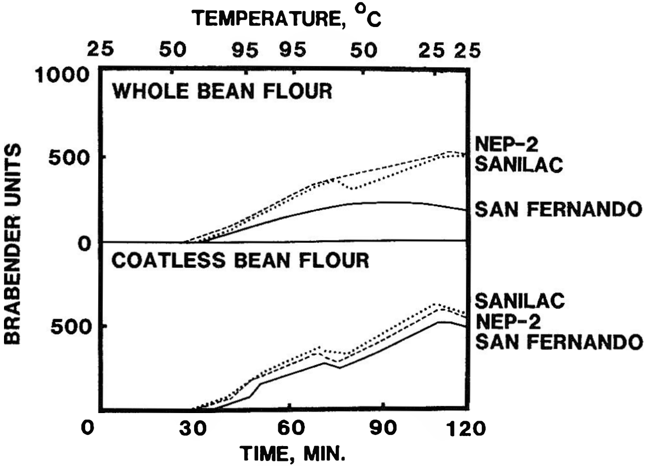

Fig. 2—Gelatinization profiles of whole bean flour and decorticated bean flour of the cultivars Sanilac, Nep-2 and San Fernando (averages of three crop years; only two years for Nep-2).

and Maywald, 1968; Vose, 1980). Both flours examined displayed increased viscosity upon cooling, indicating retrogradation of the starch component.

The coatless bean flours reached higher viscosity levels than the whole bean flours. The San Fernando flour pastes were not as thick as those of the other two cultivars. The Nep-2 whole bean flour paste was slightly more viscous than the Sanilac whole bean flour paste, but this relationship was reversed for the decorticated bean flours.

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PHOTOACOUSTIC ASSESSMENT OF COFFEE BEANS. . .FROM PAGE 849

from compounds such as chlorogenic acids to the whole coffee PA spectrum peak at 360 nm should be considered.

The literature reports (Clifford, 1975a,b) on the strong thermal lability of chlorogenic acids, with decomposing thresholds at 220°C. We have determined spectral variations induced in coffee sample PA spectrum by roasting of the coffee beans for different amounts of time (Fig. 2). The gradual diminution of the observed broad 360 nm peak in the PA spectra of these coffee samples still precludes a clear cut link to the chlorogenic acids. However, we feel that the gradual disappearance of this peak as a function of the roasting time of the coffee beans, at 230°C, may be an indicator of the significance of the water-soluble components responsible for it.

In summary, nondestructive determination of the PA spectrum of freshly ground green coffee samples has a potential application in the assessment of water-soluble components in the samples. The simplicity of the PA technique offers an attractive alternative to standard elaborate chemical and physical procedures to obtain equivalent information and enables the study of the coffee components under essentially the same conditions as those prevailing in the intact beans. This fact may become of major importance when comparing coffee beans from different cultivars or different processing procedures (Carvalho, 1962; Oliveira et al., 1972).

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A Research Note

Changes in Cucumber Volatile Compounds on Chilling Temperature and Calcium Chloride Treatment

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ABSTRACT

The effects of chilling and $CaCl_2$ treatment on volatile compounds of European cucumber fruit were studied. European cucumbers were treated with/without $CaCl_2$, stored at 4.4°C and held at 20°C. Their volatile compounds were isolated and analyzed. Lauric, stearic and oleic acids decreased, whereas linoleic and linolenic acids increased by chilling and $CaCl_2$ treatment. Major volatile compounds were identified, trans-2-cis-6-nonadienal and trans-2-nonenal decreased, while hexanal, trans-2-hexenal and 2-pentylfuran increased on chilling and $CaCl_2$ treatment. The 6-nonenal and tetradecanal showed no clear trend.

INTRODUCTION

ALTHOUGH LIPIDS in cucumbers occur only to the extent of 0.10% (Salunkhe, 1974; Peng and Geisman, 1976; Yamaguchi, 1983), the characteristic flavor compounds of this fruit are derived enzymatically from linoleic and linolenic acids when the tissues are disrupted (Litman and Numryck, 1978). The enzyme systems responsible for the conversion of the unsaturated 18-carbon fatty acids to the 6- and 9-carbon carbonyl compounds of cucumber volatiles are believed to be composed of 4 enzyme systems: a lipoxygenase, a hydroperoxide cleaving enzyme (lyase system), an isomerase and an oxidoreductase (Galliard and Phillips, 1976; Galliard et al., 1976; Phillips and Galliard, 1978; Hatanaka et al., 1975; Tressl et al., 1981).

Chilling injury in sensitive commodities is generally believed to cause a change in the physical state of the membrane (Lyons and Raison, 1970). This has led some investigators to relate chilling sensitivity or tolerance with changes in fatty acid composition, particularly linoleic and linolenic acids (St. John and Christiansen, 1976; Wang and Baker, 1979). One of the approaches to alleviate chilling injury is the application of chemicals (Scott, 1975; Schiffmann-Nadel et al., 1975; Sasson and Bramlage, 1981). Calcium chloride has been shown to reduce chilling injury in tomatoes (Murphy and Evensen, 1982), cherries (Lidster et al., 1979), avocados (Chaplin and Scott, 1980) and okra (Ilker and Morris, 1975). The purpose of this study was to determine the effects of the application of $CaCl_2$ on volatile compounds of European cucumber fruit, which had been stored at 4.4°C.

MATERIALS AND METHODS

Samples

European cucumber, cv. 'V-5' (*Cucumis sativus* L.) was grown in the Ohio State University Horticultural greenhouses using standard cultural practices. The fruits were harvested when they reached 25–30 cm in length and 4–5 cm in diameter.

Calcium chloride treatment

Eight cucumbers were submerged in 6% $CaCl_2$ solution (w/v) for 30 min; control fruits were dipped in distilled water only.

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Table 1—Effect of chilling and $CaCl_2$ treatment on fatty acid compostion of total lipids of cucumber fruit[z]

Fatty acid[y]	Treatment[x]		
	Control, unchilled	Control, chilled 0% $CaCl_2$	Chilled 6% $CaCl_2$
12:0	1.4 a	0.6 b	0.1 c
14:0	2.0 a	1.7 a	0.2 b
16:0	34.2 a	38.3 a	34.0 a
18:0	6.7 a	3.3 b	1.7 c
18:1	5.9 a	3.6 b	3.8 b
18:2	23.8 b	25.1 b	29.7 a
18:3	18.4 b	21.5 b	30.6 a

[z] Means of three determinations expressed as % of total fatty acids
[y] Carbon number: number of double bonds
[x] Means within the same row followed by the same letter are not significantly different ($P < 0.05$)

Chilling storage

After treatment, fruits were allowed to air dry at room temperature (20°C), placed in polyethylene film liners with tops loosely tied, and stored at 4.4°C for 7 days. They were then removed from cold storage and transferred to 20°C for another week. Unchilled and untreated fruits were maintained at 20°C for 14 days as controls. Volatile compounds were analyzed at 0, 5 and 7 days at 20°C.

Fatty acid analysis

Triplicate 200g samples of peeled cucumber tissue were boiled 5 min. in isopropyl alcohol (Kates, 1972). Distilled water (200 mL) was added and the sample blended for 2 min in a Waring Blendor. Total lipids were extracted according to the method Peng (1975). Extracted lipids were converted into fatty acid methylesters (Metcalfe et al., 1966) and analyzed with a Hewlett-Packard model 5580A gas chromatograph equipped with a flame ionization detector and electronic integrator, using a stainless steel column (305 cm × 0.3 cm OD) packed with 10% by weight diethyleneglycol succinate (DEGS) on acid-washed Chromosorb W, 80–100 mesh support (Supelco, Inc., Bellefonte, PA). Oven temperature was 190°C; injection temperature, 200°C; detector temperature 250°C and carrier gas (nitrogen) at a flow rate of 20 mL/min. Identifications of the fatty acids on the chromatograms were made by comparing retention time with those of reference compounds. Fatty acids were expressed as areas percentage of the total area from all methylesters.

Volatile compounds

Peeled fruits were sliced and duplicate 300 g samples were frozen immediately in liquid nitrogen. Frozen samples were blended with 300 mL distilled water for 2 min and distilled in Likens-Nickerson apparatus with 50 mL of hexane for 1.5 hr. The hexane extract was dried over Na_2SO_4, and the solvent was removed under reduced pressure with a stream of N_2 to obtain a concentrate of 0.5 mL. Volatiles were analyzed with a Hewlett-Packard model 5880A gas chromatograph using a 10% of DEGS column as described previously under the following operating conditions: programmed oven temperature from 100–180°C at an increment of 4°C/min. rate, injection temperature 200°C and detector temperature 250°C and carrier gas (nitrogen) at 20 mL/min. flow rate. The aldehyde standards trans-2-cis-6-nonadienal, trans-2-nonenal and cis-6-nonenal (ICN Pharmaceuticals, Inc., Plainview, NY), were used to compare retention times. Compounds whose standards were not available were identified by gas chromatography-mass spectrometry (GC-MS) in a Finnigan 4021 GC-MS data system.

Table 2—Volatile compounds of unchilled, untreated and chilled, $CaCl_2$-treated cucumber fruit[z]

Compound	Unchilled, untreated	Days at 20°C: 0 Control	0 6%$CaCl_2$	5 Control	5 6%$CaCl_2$	7 Control	7 6%$CaCl_2$
Hexanal	0.4	3.5	2.2	7.8	7.1	5.8	7.1
2-Pentylfuran	1.6	2.2	2.0	6.0	7.5	5.6	6.9
2-Hexenal	0.6	1.9	1.1	7.1	5.1	5.1	5.8
6-Nonenal	1.1	1.0	2.3	0.8	1.1	0.9	0.8
trans-2-Nonenal	15.9	13.2	9.1	6.5	7.1	7.1	8.3
trans-2-cis-6-Nonadienal	39.1	20.7	26.4	19.4	14.7	18.5	21.8
Tetradecanal	7.1	11.2	1.6	3.9	8.1	5.8	5.1

[z] Mean of two determinations expressed as % of total volatile compounds

RESULTS & DISCUSSION

The fatty acid composition of the crude lipids in control and treated fruits is shown in Table 1. Chilling and $CaCl_2$ treatment decreased lauric, stearic and oleic acids. Palmitic acid was not significantly different in chilling and $CaCl_2$ treatment. Application of $CaCl_2$ to chilled fruits increased the amounts of linoleic acid (25.1 to 29.7%) and linolenic acid (21.5 to 30.6%). Research on polar lipids in cucumber fruit (Wang and Baker, 1979) demonstrated an increase in linoleic and linolenic acids on chilling and treatment. This study found an increase of these two fatty acids in total cucumber lipids upon chilling and $CaCl_2$ treatment.

Gas chromatographic analyses of European cucumber volatile compounds are presented in Table 2. The major components were found to be trans-2-cis-6-nonadienal and trans-2-nonenal which were in agreement with results reported in cucumbers (Forss et al., 1962; Grosch and Schwarz, 1971; Kemp et al., 1974; Hatanaka et al., 1975; Phillips and Galliard, 1978; Tressl et al., 1981). Tetradecanal, 2-pentylfuran and 6-nonenal were the minor volatile compounds. Tetradecanal and 6-nonenal were reported by Kemp et al. (1974) from cucumber essence, and 2-pentylfuran was identified by Tressl et al. (1981) from cucumber homogenates. There were two unidentified peaks on the fresh cucumber extract GLC chromatogram, however, they disappeared after chilling and $CaCl_2$ treatment. The volatile compounds observed in chilled and/or treated fruits showed the same pattern as those in fresh fruits. The enzymic formation of aldehydes and other carbonyl compounds from linoleic and linolenic acids on disruption of plant tissues is well documented (Grosch and Schwarz, 1971; Litman and Numrych, 1978; Tressl et al., 1981).

There was a significant decrease in trans-2-cis-6-nonadienal (47%) and trans-2-nonenal (17%) on chilling. These differences were more substantial in fruits chilled 5 and 7 days after holding at 20°C. The decrease in volatile concentration was lowest in fruits chilled 5 days after holding at 20°C. This decrease was accompanied by a slight increase in 2-pentylfuran, a twofold increase in 2-hexenal and almost sevenfold increase in hexanal, and all reached a peak at 5 days after holding at 20°C (3 to 16-fold increase). There was no clear trend in tetradecanal and 6-nonenal.

The comparison between $CaCl_2$-treated and chilled and untreated-unchilled fruits also revealed changes in volatile compounds. Hexanal, 2-hexenal and 2-pentylfuran were significantly increased after $CaCl_2$ treatment and reached eighteenfold, eightfold, and fourfold increase, respectively, after 5 days holding at 20°C, while trans-2-cis-6-nonadienal and 2-nonenal were decreased with the same pattern. Tetradecanal and 6-nonenal were varied. Trans-2-cis-6-nonadienal was higher in treated fruits than in control fruits at 0 and 7 days after holding at 20°C. It has been shown that hexanal is derived primarily from linoleic acid while trans-2-cis-6-nonadienal comes from linolenic acid (Grosch and Schwarz, 1971; Tressl et al., 1981). Since $CaCl_2$ application increased linoleic and linolenic acids it can be postulated that the changes in the levels of these two compounds were the consequences of the alteration of these two fatty acids.

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A Research Note

Continuous Hydrolysis of Fructans in Jerusalem Artichoke Extracts using Immobilized Nonviable Cells of *Kluyveromyces marxianus*

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ABSTRACT

Non-viable immobilized cells of *Kluyveromyces marxianus* in alginate beads having inulase (β-2,1-fructan fructano-hydrolyase E.C.3.2.1.7) acitivity were used as biocatalyst in a packed bed reactor. Extracts of Jerusalem artichoke tubers were contacted with the biocatalyst for continuous conversion of the fructan component to fructose. In a bed reactor packed with 100 mL of beads, a volumetric productivity of 136 g/L/hr total reducing sugars was obtained with 98% substrate conversion. When operated continuously for 30 days, a 55% loss in the original activity was observed, giving a half life for the biocatalyst of 28 days.

INTRODUCTION

FOR A LONG TIME, the commonly used sweetner in the food industry has been sucrose. For medical and nutritional reasons, D-fructose has gained tremendous popularity in recent years as a sweetening agent in both the food and beverage industries (Scheinin et al., 1975; Sharman, 1976; Fleming and GrootWassink, 1979). High fructose containing syrups (HFCS) are currently manufactured from corn starch by hydrolysis and subsequent enzymatic isomerization to give a fructose-glucose blend (Bucke, 1980). Ion-exchange techniques have been developed for enrichment of fructose and sucrose derived invert syrups to give syrups with a D-fructose content of over 90%, the so-called Ultra High Fructose Glucose Syrups (UHFGS) (Fleming and GrootWassink, 1979).

Increase in the use of UHFGS has prompted an extensive evaluation of an alternative technology and use of naturally occurring D-fructans as substrate for the production of such syrups. The D-fructans most commonly considered are the inulin types found in Jerusalem artichoke, chicory and dalhia (Fleming and GrootWassink, 1979). The hydrolysis of inulin sugar from Jerusalem artichoke produces syrups with D-fructose content over 75% (Yandamme and Deryke, 1983). Chemical hydrolysis of inulin to fructose displays several drawbacks and this has forced interest towards the use of enzymatic hydrolysis using inulases (GrootWassink and Fleming, 1980; Yandamme and Deryke, 1983). Hydrolysis can be performed by the free enzyme (Guiraud and Galzy, 1981), or immobilized enzyme (Kim et al., 1982) as well as by immobilized whole cells (Guiraud et al., 1983).

Kluyveromcyes marxianus was found to produce inulase (β-2,-1-fructan fructanohydrolase E.C.3.2.1.7) with a high activity towards sucrose and inulin (Parekh and Margaritis, 1985). As much as 78% of the total inulase was found to be cell bound. Hydrolysis of fructan is a single bioconversion reaction, and a continuous basis requires the use of immobilized

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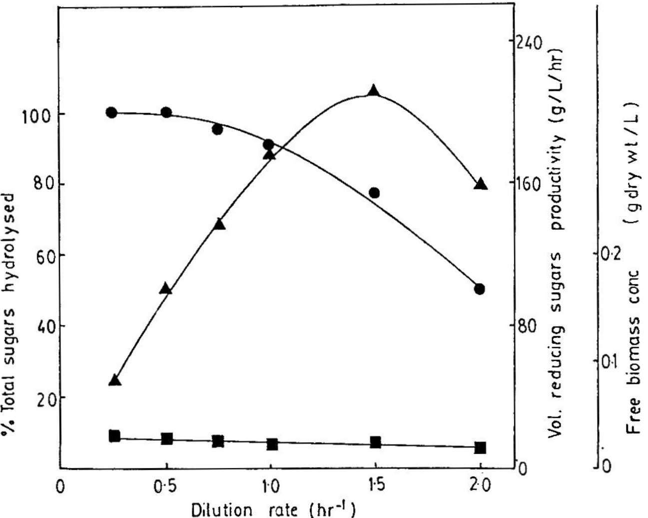

Fig. 1—Hydrolysis of fructans of Jerusalem artichokes as a function of dilution rate in a packed bed column reactor containing immobilized cells of K. marxianus: (●) % sugars hydrolysed; (▲) vol. reducing sugars productivity; (■) free biomass.

inulase. This requirement prompted the evaluation of the use of heterogeneous non-viable whole cell biocatalyst preparation. The use of a nonviable biocatalyst offers a number of advantages: (a) no extraction or purification of inulase; (b) greater reactor stability; (c) facilitated transfer of substrate to and from enzyme location due to mildly ruptured or permeabilized dead cells; and (d) unused substrate can be recycled. A review of the use of non-viable cells in general and its industrial application has appeared (Gestrelins, 1984).

Systemic literature surveys revealed no published information and data on the use of immobilized non-viable biocatalyst for hydrolysis of the fructan-containing extracts of Jerusalem artichoke. The objective of this study was to develop conditions for generating non-viable cells of *K. marxianus* containing inulase and to use immobilized whole cells for industrial application.

MATERIALS & METHODS

Preparation of substrate

The inulin type sugars from Jerusalem artichoke tubers were extracted as described by Margaritis and Bajpai (1982). The extract was concentrated under vacuum to approximately 280 g/L total carbohydrates. The proteins from the turbid extract were removed by lowering the pH to 4.3 with 2N sulphuric acid and holding for 2 hr at 50°C. After filtration, the pale yellow supernatant obtained was adjusted to pH 5 with lime.

Micro-organisms and growth conditions

Kluyveromyces marxianus UCD (FST) 55-82 was grown in 2L Erlenmeyer flasks. The medium contained 10g inulin and 5g yeast

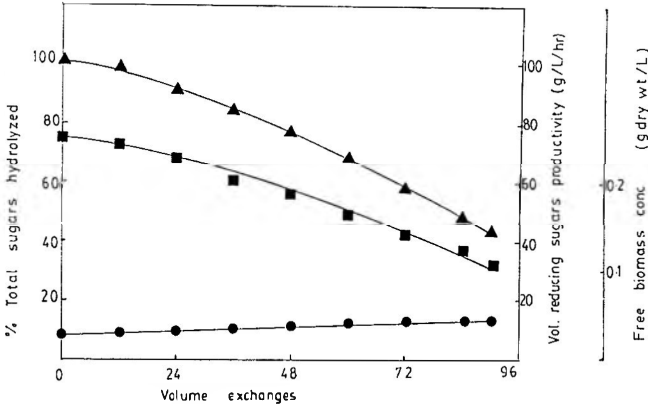

Fig. 2—Continuous hydrolysis of fructans during operation at a fixed flow rate (15 mL/hr) in a packed bed column reactor: (▲) % sugars hydrolysed; (■) vol. reducing sugars productivity; (●) free biomass.

extract per liter tap water at pH 5.0. Cells were harvested after 18 hr, washed in 0.1M acetate buffer, pH 5, and concentrated by centrifugation at 3000 rpm for 10 min (10 times its original concentration).

Preparation of nonviable biocatalyst

The thick slurry of yeast cells was suspended in 95% ethanol in the ratio 1:10. The solvent-cell mixture, in 2L conical flasks, was agitated at 220 rpm (25°C). Viability of the yeast cells was periodically checked by plate counts. After all the cells had been killed they were centrifuged and washed twice with sterile 0.1M acetate buffer. The dead cells were then immobilized in 1.8% alginate beads as described earlier (Margaritis and Bajpai, 1982).

Inulase assay

Inulase activity of the immobilized preparation was measured by determining the reducing sugars released from inulin by Somogyi's method (1952). Five mL 4% (w/v) inulin in 0.1M acetate buffer, pH 5, and 1 mL immobilized bead preparation were incubated for 10 min at 50°C. Periodically, samples (0.1 mL) with withdrawn and assayed for total reducing sugars. One unit of inulase activity is defined as the production of 1 μmole hexose per minute. The immobilized preparation contained 994 units inulase activity per gram dry weight cells.

Immobilized cell bioreactor system

The reactor column consisted of a borosilicate glass tube (1.8 cm i.d. and 15 cm long) fitted with a water jacket. The total working volume of the bioreactor was 130 mL, and the column was packed with 100 mL beads loaded to a cell density of 56g dry wt/L bead volume. A positive displacement peristaltic pump (Sybron-Brickman model 1P-12) was used to vary the liquid feed flow rate in the ascending manner (20–80 mL/hr ± 0.3 mL). The feed was sterilized at 121°C for 15 min in 20L carboy bottles. Total sugars in the feed were determined by the anthrone method (Scott and Melvin, 1953). Effluent samples were collected at each dilution rate every 3 hr and assayed for total reducing sugars (Somogyi, 1952), glucose (Bergmeyer et al., 1974) and free cell concentration in the liquid phase. A steady state was assumed when the level of total reducing sugars was constant in 3 successive samples. A temperature of 50°C was maintained during the operation of the reactor. During the study the reactor activity remained unaltered with time.

RESULTS & DISCUSSION

INCREASING the dilution rate over 1.5 hr^{-1} gave rise to a decrease in the reducing sugars productivity and an increase in the unhydrolysed substrate (Fig. 1). Complete conversion of 20% of substrate solution was realized at dilution rates below 0.5 hr^{-1}, corresponding to a mean residence time of 2.3 hr. There was no significant leakage of free cells from the beads, eliminating cell recovery requirements. Also, the low cell release from the beads minimized the contamination of the product and did not lower the catalytic capacity and efficiency of the reactor. The hydrolysed tuber extract contained D-fructose and D-glucose in a 3:1 ratio, thus, the average polymerization rate of extracted fructans was particularly low.

Operational stability

The packed bed was run continuously for 30 days at a constant dilution rate of 0.37 hr^{-1} (flow rate-15 mL hr^{-1}). The volumetric reducing sugars productivity remained constant (Fig. 2) at 74 g/L/hr for 24 volume exchanges (4 days), but thereafter decreased steadily. There was no significant increase in the effluent biomass during the 30 days of operation and during this period 55% loss in the catalytic activity occurred (90 volume exchanges), corresponding to a half life of 28 days. Using viable immobilized whole cells of *K. marxianus*, a half life of 22 days was obtained by Kierstan and Bucke (1977).

This study represents a novel biotechnological approach of fructan hydrolysis into high fructose solution by use of immobilized dead cells. Two points emerge from this study. First, substrates such as the fructans of the agricultural products currently underutilized (Fleming and GrootWassink, 1979) could easily be adapted as inexpensive feedstock for economic production of high fructose. The fructose content obtained by this biotransformation technique was higher and its production cost relatively lower if the plants that are easy to grow, e.g. Jerusalem artichokes and chicory, and that give high yields per hectare are used. Second, under the operational conditions described, the bioconversion of inulin sugars to fructose could be carried out effectively in a packed bed bioreactor; the column operation did not cause any microbial contamination nor any biomass leakage, thus saving cost in downstream processing.

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A Research Note

Nitrogen Determination of Commercially Prepared Lactalbumin

K. R. AIYAR, R. I. W. GREIG, and M. SANGROUBER

ABSTRACT

Commercially produced denatured whey protein (lactalbumin) was analyzed for total protein using the macro-Kjeldahl technique. The effect of catalyst ($CuSO_4$, HgO) and preincubation on total nitrogen was determined. Results showed that HgO gave consistently higher and more reproducible results than $CuSO_4$. Preincubating the sample in concentrated H_2SO_4 for 24 hr prior to digestion improved the recovery of nitrogen.

INTRODUCTION

THE MAJOR PROTEIN constituents of whey are β-lactoglobulin, α-lactalbumin, bovine serum albumin, immunoglobulins and proteose-peptones. Also present are several minor whey proteins (Marshall 1982). The commercial product lactalbumin is a heat denatured mixture of several of the above proteins, the heat denaturation occurring under acid conditions. New Zealand produces significant quantities of lactalbumin, the process involving heating the whey at pH 4.6 to temperatures of 95°C for a nominal holding time of at least 30 min. The fine precipitated protein is centrifugally separated, washed once and reseparated (14–16% solids) prior to spray drying. Despite its insolubility, the nutritive value of lactalbumin is high and finds use in nutritional supplementation of non meat-based foods (Greig, 1979). The water-holding properties also cannot be discounted particularly in sausage or meat loaf type products, as recent unpublished research work in this department has shown. (Greig, 1984).

The current procedure for analysis of nitrogen in commercially produced lactalbumin is the same as that used for acid casein. The approach disregards the fundamental differences between the two products, both in terms of manufacturing practice and protein type. The effect of severe process conditions on the whey protein is unclear, except for the probable sulphydryl-disulphide interchange accompanying the heat denaturation. This is particularly so in the area of potential heat-induced hydrolytic cleavage or sensitization of peptide bonds and covalent bonds in general.

The Kjeldahl procedure is a very harsh treatment and over the years, various attempts have been made to modify the methodology to reduce potential loss of nitrogen (Fisher, 1983). Unfortunately, the effects of protein processing conditions on the analysis of nitrogen have not been reported. In this study the effect of catalyst and digestion time on the recovery of protein nitrogen was determined.

MATERIALS & METHODS

COMMERCIALLY PRODUCED lactalbumin was purchased from the N.Z. Dairy Board, Wellington. Total protein was determined by the Macro Kjeldahl digestion method (Dairy Division Manual, 1983) using the following quantities: potassium sulphate, 20g; sulphuric acid (1.84 g/mL), 40 mL; copper sulphate, 0.2g; lactalbumin, 1g (approximately); digestion time, 90 min after clearing. Where mercuric oxide was used as a catalyst instead of copper sulphate, the AOAC recommendation of 0.7g was followed (Fisher, 1983). The digest, after cooling was diluted to 250 mL with distilled water, 130 mL 50% sodium hydroxide added and the ammonia was distilled into 40 mL 4% boric acid solution (150–200 mL distillate). Where mercuric oxide catalyst was used, 15 mL 15% sodium hypophosphite was added with the sodium hydroxide solution. The indicator was a mixture of 0.2g methyl red in 50 mL ethanol and 0.04g methylene blue in 50 mL water. The back titration was done with standardised 0.1M hydrochloric acid. Estimated nitrogen was converted to total crude protein with the factor 6.38. Preincubation of samples was carried out by adding the sulphuric acid to the sample of lactalbumin in the digest vessel, stoppering the flask and holding for 24 hr at 25°C prior to digestion.

All chemicals and reagents used were of A.R. quality and results were expressed to two decimal places. Statistical analyses of results were conducted by Analysis of Variance using the Minitab statistics package (Massey University).

RESULTS & DISCUSSION

PRELIMINARY WORK with both lactalbumin and ammonium sulphate as a standard, showed that there were obvious differences in the total estimated nitrogen content in relation to the catalyst used. Mercuric oxide catalyst gave consistently higher nitrogen results than did copper sulphate (Table 1).

These results show that not only did mercuric oxide catalyst result in higher nitrogen values than copper sulphate, but that preincubated samples gave significantly higher results than those digested immediately after adding sulphuric acid. From Table 1, the average total protein using copper sulphate was 83.84% whereas the average on the preincubated samples (copper sulphate) was 85.78%, an increase of nearly two percentage points. Figures for mercuric oxide showed the same trend but at a higher level (84.5 and 87.0%, respectively). Significant differences were observed at the 1% level of significance for all results.

Results given in this paper clearly establish the superiority of mercuric oxide over copper sulphate in providing consistently higher nitrogen results in both normal and preincubated lactalbumin samples. The New Zealand Dairy Division manual of *Standard Laboratory Methods* (Dairy Division, 1983) states that "for environmental and safety reasons, the mercuric oxide catalyst can be replaced by copper sulphate, provided an af-

—*Continued on page 858*

Table 1—Effect of catalyst type and preincubation of the digest mixture (24 hr) on the estimation of total protein in commercial lactalbumin

	Total crude protein percent			
	Copper Sulphate		Mercuric oxide	
	Normal	Preincubated	Normal	Preincubated
	83.18	86.19	84.49	87.22
	84.03	86.11	83.89	87.23
	83.95	85.98	84.61	87.29
	83.96	85.74	84.66	87.45
	83.97	86.23	84.73	87.38
	84.06	85.18	84.62	86.59
	83.99	85.35	84.59	85.65
	83.73	85.01	84.21	87.53
	83.54	85.73	84.61	87.38
	83.99	86.23	84.55	86.39
Avg	83.84 ± 0.28[a]	85.78 ± 0.46	84.50 ± 0.25	87.0 ± 0.61

[a] Average ± standard deviation

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A Research Note

High Performance Liquid Chromatographic Analysis of Riboflavin (Vitamin B_2) with Visible Absorbance Detection in Italian Cheeses

B. STANCHER and F. ZONTA

ABSTRACT

A high-performance liquid chromatographic method for riboflavin determination in cheese samples was developed. Reverse phase conditions were used, i.e., a C-18 column isocratically eluted with an acetonitrile water mixture (20:80). A shift of the detection wavelength from 267 to 446 nm was proposed as a simple and very effective means for circumventing the problem of incomplete resolution of riboflavin from interfacing peaks. The detection limit of riboflavin was 2.5 ng, a value well above the level required for determining the riboflavin content in cheese samples. From both high- and low-fat cheese, the riboflavin recovery was the same (90.2%). The riboflavin content of several different Italian cheeses was determined.

INTRODUCTION

RIBOFLAVIN in foods was recently determined, using HPLC, by several authors (Augustin, 1984; Mauro and Wetzel, 1984) and particularly Ashoor et al. (1983) who published an applicative method for eggs and dairy products.

In our case, results very close to those of Ashoor et al. (1983) were obtained when analyzing cheese samples, but this method could not be applied to every type of cheese. In several cases, the resolution of the riboflavin peak was reduced or lost, owing to the interference of coeluting substances. The purpose of this study was to modify the above-mentioned method to render it suitable for the riboflavin analysis of any type of cheese.

MATERIALS & METHODS

THE CHROMATOGRAPHIC APPARATUS consisted of a pump module (Series 10 liquid chromatograph, Perkin Elmer, Norwalk, CT), a variable-wavelength spectrophotometer (LC 75, Perkin Elmer) and a data processor (Chromatopac C-R1B; Shimadzu, Kyoto, Japan). A guard column (5 × 0.4 cm i.d.) (Supelco, Bellefonte, PA) dry-packed with 40-μm C-18 pellicular packing was connected to the analytical column (25 × 0.4 cm i.d.) (LiChrosorb RP 18, 5 μm, E. Merck, Darmstadt, F.R.G.). A UV-visible spectrophotometer (SuperScan Model 3, Varian Techtron, Springvale, Australia) was also used for determining the concentration of the riboflavin standard solution.

Acetonitrile (HPLC grade, Carlo Erba, Milan, Italy) and quartz bidistilled water were used for preparing the mobile phase. Methanol and glacial acetic acid were from Riedel de Haën (Seelze, F.R.G.).

Cheese samples were purchased at random in grocery stores.

A standard solution was obtained by dissolving 40.0 mg of riboflavin (Fluka, Buchs, Switzerland) in a 1000-mL amber-colored volumetric flask using bidistilled water. A fivefold diluted standard solution was later obtained and its concentration was controlled by recording the absorbance spectrum. The absorbance values obtained at 267 nm and at 446 nm gave log E values identical to those previously reported (Phillips and Nachod, 1958–59). This diluted standard solution was used to obtain the calibration curve.

To plot one standard addition curve, three identical cheese samples were separately spiked with 200, 400 and 600 μL, respectively, of the standard solution before being analyzed simultaneously with an unspiked sample, as described below.

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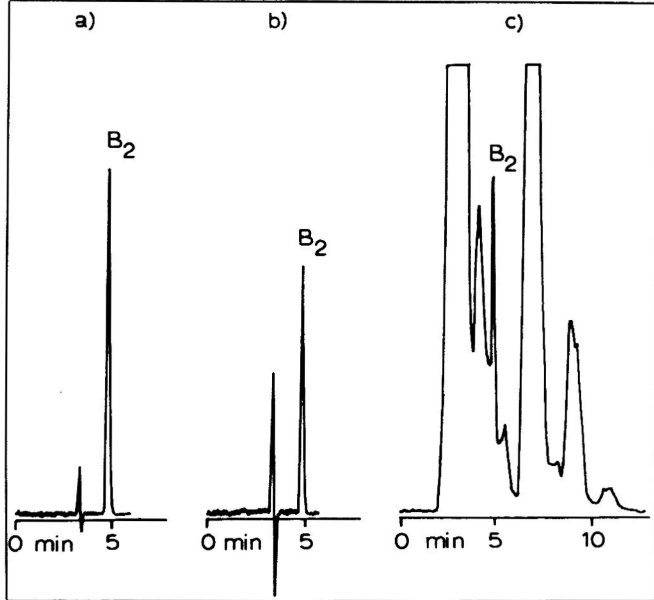

Fig. 1—Chromatogram of (a) riboflavin standard, 5 μL (40 ng), and of (b) riboflavin extracted from Parmigiano reggiano cheese, 25 μL. Detection wavelength 446 nm. In (c) an injection of the same cheese sample, 10 μL, is detected at 267 nm. Mobile phase: acetonitrile:water (20:80 v/v), flow rate 1 mL/min.

A slightly modified method, derived from that described by Ashoor et al. (1983), was used for obtaining HPLC samples from cheese. A (5.0 g) sample of cheese, ground in a food chopper or cut very finely, was weighed in a centrifuge tube (9 × 2 cm I.D.). Seven milliliters of a methanol-water solution (1:2) were added, and the mixture was homogenized (Virtis, Gardiner, New York, NY) for 4 min; 3 mL of glacial acetic acid were then added, and, after stirring again, the suspension was centrifuged for 15 min at 2000 × g. The aqueous layer was transferred to a 20-mL volumetric flask, and the precipitate was washed three times with 4-mL aliquots of a water-methanol-acetic acid (glacial) solution (65:25:10). The volumetric flask containing the pooled aqueous layers was then filled up to the mark, and its content was centrifuged again. An aliquot of the clear supernatant (about 500 μL) was filtered into a 1-mL amber flask using a 0.45 μm pore size Millipore-HV_4 Filter (Nihon Millipore, Kogyo K.K. Yonezawa Japan). Twenty-five microliters of the solution were used for the analysis.

RESULTS & DISCUSSION

WHEN THE HPLC METHOD (UV detection at 270 nm) proposed by Ashoor et al. (1983) was first applied to a sample of Asiago cheese, a chromatogram in close agreement with that reported in the case of cottage cheese was obtained. Things changed when Parmigiano reggiano was analyzed in the same manner: interfering peaks of the matrix tended to overlap and mask the riboflavin peak, hampering its quantitative determination. In such cases fluorescence detection may prove useful. A spectrofluorimetric method was developed by Rashid and Potts (1980) for determining riboflavin in milk without using

Table 1—Riboflavin concentration in Italian cheeses

Cheese[a]	Riboflavin[b]	Std. dev.	C.V.%[c]
Fontina	252	10.5	4.0
Gorgonzola (dolce) (mild)	296	13.5	4.6
Grana padano	248	10.5	4.2
Parmigiano reggiano	358	9.5	2.6
Pecorino romano	117	6.0	5.1
Pecorino siciliano	257	9.0	3.5
Asiago	240	15.5	6.5
Caciocavallo	237	6.5	2.7
Fiore sardo	375	8.0	2.1
Montasio	305	10.5	3.4
Pressato	n.a.[d]		
Provolone (piccante) (strong)	295	7.0	2.4
Ragusano	n.a.		
Taleggio	195	9.5	4.9

[a] Cheeses listed are those required to meet certain commodity standards defined by the Italian law (Gazzetta Ufficiale, 1955). The law does not specify any level for riboflavin in cheeses.
[b] Average of three determinations expressed in μg per 100g fresh cheese.
[c] C.V. coefficient of variance.
[d] n.a. = cheese sample not available.

HPLC; but Augustin (1984), who used the fluorescence detector for the HPLC analysis of the vitamins B_1 and B_2 in foods, still reported incomplete separation of riboflavin from interfering peaks.

To avoid interferences, in our HPLC system the absorbance detection wavelength was set at the secondary maximum of riboflavin, 446 nm, and, as a result, in the chromatogram (Fig. 1b) no other peaks could be observed.

Relative to the detection at 267 nm, the absorbance was reduced by a factor of 2.6; nonetheless the detection limit of the riboflavin peak was 2.5 ng. Previously reported detection limits for riboflavin were 0.1 ng (fluorescence) and 5 ng (UV, 254 nm) (Augustin, 1984). In cheese, riboflavin was present in relatively high concentration (at least 100 μg/100 g), and therefore, the detection limit was not a critical factor.

Figure 1 shows the chromatogram obtained with an injection of the riboflavin standard, 40 ng, at 446 nm (a) and the chromatogram obtained from Parmigiano reggiano cheese at 446 nm (b) and 267 nm (c). By spiking the cheese sample with riboflavin the peak height increased.

The calibration curve and the curves obtained by means of the standard addition method applied to three different cheeses (with low, medium and high-fat content) were constructed. By dividing the slope of the standard addition curves by that of the calibration curve, recovery by the analytical procedure was obtained. The recovery was practically the same in the case of the three different cheese samples tested, its mean value being 90.2%.

The riboflavin concentrations found in Italian cheese samples are reported in Table 1.

The method described is practical for routine analysis and it is planned to use it to try to correlate the vitamin content variations in cheeses with the seasonal variations in milk or, when testing different types of cheese, to correlate vitamin differences with different production techniques.

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terboil period of 90 min is used — the result so obtained is comparable with the reference method." Our results do not substantiate this. Results given by other workers (Potts et al., 1947; Miller and Houghton, 1945) working on fish meal and β-lactoglobulin also indicate that mercury should be the choice when compared with copper.

The results obtained have surprised us and work is continuing on the Kjeldahl technique with reference to the preincubation aspects, digestion times, catalyst mixtures for other foodstuffs. The Kjeldahl method suffers from various difficulties which center around the problem of digesting the mixture completely without loss of nitrogen in the process. A great many different modifications of the Kjeldahl techniques have been suggested in the literature, particularly in the use of the catalyst to accelerate the digestion time.

Variations in results have significance in two important areas. Firstly, sophisticated instruments have been developed for protein analysis, but these instruments need to be developed against the reference Kjeldahl method. Secondly, export markets demand 90% total protein in lactalbumin on a dry matter basis (Aiyar, 1984). Results obtained (Table 1), when converted to a dry basis show 88.5% protein using $CuSO_4$ and 91.87% protein (preincubated HgO) based on 5.3% water content in the powder. Powders which would normally be downgraded or not accepted as export quality on the basis of a Kjeldahl determination with $CuSO_4$ as catalyst would easily pass export standards when tested by Kjeldahl using HgO as catalyst.

On the basis of these results, it is recommended that, mercuric oxide catalyst and a preincubation step of 24 hr should be used for determination of protein in lactalbumin.

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A Research Note

Electromyography as a Novel Method for Examining Food Texture

M.M. BOYAR and D. KILCAST

ABSTRACT

Integrated electromyographic (EMG) data from the masseter muscle were found to be related to the oral force exerted by the muscle during activity. The height of integrated EMG waves recorded on chewing gelatin and carrageenan gels revealed distinct differences throughout the chewing process, which assisted in the interpretation of previously reported subjective variations between these two gel systems.

INTRODUCTION

FOOD TEXTURE is an important sensory attribute of food which often determines quality and acceptability. Electromyography (EMG) is providing a novel way in which to examine food texture. The technique gives a direct measure of muscle activity (Lehman and Ritchie, 1970), such as that required for chewing food. This activity signal represents electrical potentials produced by the muscles, and provides the brain with some of the necessary information to interpret texture.

Conventional instrumental techniques, (e.g., Kilcast et al., 1984) which do not satisfactorily mimic such factors as oral motion, rate of force application and the effect of saliva, often yield only weak correlations with subjective descriptors.

EMG offers the possibility of making physiological measurements on subjects during chewing and of bridging the gulf between traditional objective and subjective assessments. This report presents a preliminary examination of the type of information that can be obtained from the technique.

MATERIALS AND METHODS

Electromyographic Equipment

Electromyographic recordings were made using a Grass Polygraph Model 7D (Grass Instrument Company, Quincy, MA) having a regulated power supply and two DC driver amplifiers, one of which is connected to a wide-band A.C. preamplifier and integrator module. In addition, the unit contains a 2-channel chart recorder having an adjustable speed of between 2.5 mm/min and 100 mm/sec, a time marker which automatically marks 1 sec intervals on the output chart and an event marker which can be used by the subject to identify points on the output chart such as swallowing. All chart recordings have been made with the amplifier's frequency bands as wide as possible (10 Hz–40 KHz) to ensure a flat frequency-amplitude response up to 200 Hz, the maximum rate of response of the chart recorder's pens. The time base on the integrator module was set at 0.2 sec, and all recordings were made with a chart-recorder speed of 100 mm/sec.

Recording electrodes

Two Medicotest ungelled surface electrodes (Cambmac Instruments, Ltd., Cambridge, England) were used to obtain EMG information from the masseter muscle, the main chewing muscle. The skin was cleansed with alcohol and good electrical contact between skin and electrode was ensured by use of Grass electrode cream. The natural chewing side of the subject (left) was chosen for measurements. The accurate placement of electrodes was achieved by positioning them relative to a reference line drawn between the nose and left ear. Two electrodes were used, each one attached at a fixed distance on either side of this reference line to obtain the recordings. A reference electrode was placed on the left ear lobe. A two-channel chart recorder was used to record both raw and integrated data.

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a) Peanut Brittle — μV axis 0–400; Time = 1.3 sec; Arbitrary Voltage Units

b) Table Jelly — μV axis 0–400; Time = 0.6 sec; Arbitrary Voltage Units

Fig. 1—The figure represents a single burst of EMG wave activity (upper wave) together with its integral (lower wave) from the third chew during the eating of (a) peanut brittle, and (b) table jelly. The duration of the waves are indicated along the horizontal axis. The vertical axis represents the amplitude of the waves in volts (upper wave, μv/cm; lower wave, arbitrary voltage units).

Foods

Gelatin-based table jelly and peanut brittle were obtained from a local supermarket. Genugel LC1 carrageenan was prepared at 2.1% m/m such that it had the same break load as that of the table jelly, as measured using the Stevens CR Texture Analyser (C. Stevens & Son Ltd., St. Albans, Herts., England) with a hemispherically ended cylindrical probe at a speed of 30 mm/min. Samples of 18g of either gel or peanut brittle were used in each EMG experiment. The single subject used was asked to try to concentrate her chewing efforts on to the left side of the mouth. EMG recordings were continued until the food samples were swallowed.

RESULTS & DISCUSSION

EMG recordings

The raw EMG recordings represent changing electrical potentials in the muscles during chewing. The information is predominantly from the surface muscle which raises the jaw,

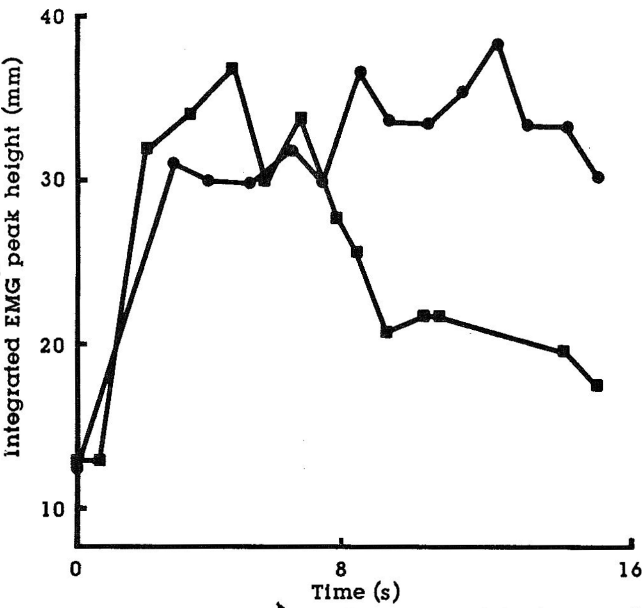

Fig. 2—The graphs represent a time course of the integrated EMG peak heights during chewing for both carrageenan (●—●) and gelatin gels (■—■) which had equivalent initial mechanical break loads. The graphs shown are typical of a set of three such experiments performed.

the masseter muscle, although deeper muscles beneath the skin electrodes contribute to the pattern. The output is in the form of a complex wave. Such bursts of activity occur during jaw closure in chewing and are spaced between periods of relative electrical inactivity during jaw opening. The integrated data represent the summated area under the raw EMG wave, reflecting the total electrical activity in the muscle. Figure 1 represents a single burst of electrical activity, together with the integral of all the waves shown. This is from the third chew during the eating of both table jelly (Fig. 1a) and peanut brittle (Fig. 1b). Product differences are evident on visual inspection of the data. By quantifying the raw or integrated data during the entire chewing process, information relating to the state of the food during chewing is clearly available.

Gels

Significant differences between gelatin and carrageenan gels can be identified from a plot of integrated EMG peak height against time (Fig. 2). Separate calibration experiments established that there was a strong relationship between integrated peak height and biting force. The two gels were chosen to have approximately equivalent break loads during penetrometry (gelatin, 880 ± 17g; carrageenan, 897 ± 49g; values represent mean values of three tests ± standard deviation). The left-hand side of Fig. 2 shows that similar oral forces were required to break the gels on the first bite. The forces observed during the chewing of gelatin increased rapidly (1–2 sec), and then fell as the gel dispersed in the saliva and was swallowed (Fig. 2). This is likely to be largely a temperature effect, since the gelatin gel will melt as it is dispersed and brought to body temperature in the mouth. The oral forces observed during the chewing of carrageenan also rose rapidly (1–2 sec). However, in contrast to gelatin, the increased force level was maintained as chewing of the gel continued (Fig. 2). This can be ascribed to a lower rate of breakdown resulting from the higher melting point of this gel.

Subjective descriptions of the different modes of breakdown of gelatin and carrageenan gels have been reported previously (Szczesniak, 1975). EMG offers a unique means of measuring the breakdown of foods *in situ*.

The curve shapes exhibited in Fig. 1 and 2 are generally similar for different subjects. Some differences can be anticipated, but preliminary observations suggested that the relative EMG outputs from different foods remained constant from subject to subject. Similarly, it has been observed that the relative output from different foods remained constant when tested on different occasions. The overall amplitude of the signals varied on different occasions (probably resulting from the need to reposition electrodes at each test session), but this potential problem could be overcome by the use of a reference food.

Further studies are being continued at the Leatherhead Food Research Association to identify and quantify measurable EMG parameters that are appropriate to specific textural types, and to examine the textural changes that occur throughout the entire mastication process. This work will be extended to the study of other muscles involved in chewing and to recording several outputs together in order to obtain information which relates more closely to the natural chewing process.

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ERRATUM NOTICE

J. Food Science 50: 1602-1606 (1985)—Formation of oligosaccharides during hydrolysis of lactose in milk using β-galactosidase from *Bacillus circulans* by Z. Mozaffar, K. Nakanishi & R. Matsuno. In the legend to Fig. 3, the key should be corrected as follows: - ● - glucose, and --- ◑ ---, disaccharide-1 (R_L = 1.2).

✓ corrected Ch. S. 4 Aug. 86

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