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Heat Transfer During the Freezing of Liver in a Plate Freezer

PHILIP G. CREED and STEPHEN J. JAMES

─────── ABSTRACT ───────

The overall heat transfer coefficient was determined for a vertical plate freezer by the transient temperature method and used in a modification of Plank's equation by Cleland and Earle to predict the freezing time of blocks of pig liver. A comparison of predicted and previously published experimental freezing times showed an average absolute error of 6.5%. Overall heat transfer coefficients for the main types of fibreboard packaging were also determined together with their effect on predicted freezing time. This work has highlighted many of the advantages of plate freezing which has yet to gain wide acceptance in the U.K. meat industry.

INTRODUCTION

APPROXIMATELY 161,000 tons of edible offal (liver, heart and kidney) are produced each year in the United Kingdom (Meat and Livestock Commission, 1984), but there is little published on its refrigeration. The largest component of edible offal is liver which for long term storage must be frozen either in air blast or plate freezers. The basic advantages of the latter method — shorter freezing times, more uniformly shaped blocks and lower energy consumption — have recently been discussed by Creed and James (1983), whose experimental results showed that liver is particularly suitable for plate freezing because its pliable nature produces good contact with the refrigerated plates. Data were obtained on the effect of block thickness (0.152, 0.102, or 0.076 m), initial temperature (5-24°C) and refrigerant temperature (−21 to −41°C) on freezing time, but the investigation covered only one packaging condition (wrapping in polyethylene film) in one particular vertical plate freezer. As the practical experimentation required to extend the data to cover other packaging conditions and plate freezing systems would have been prohibitively expensive, it was decided to seek a theoretical method that would model the existing results and use it to extend the available data.

Theoretical predictions of freezing times use either analytical or iterative mathematical models. Both methods have been extensively reviewed by Bakal and Hayakawa (1973), Kinder and Lamb (1974) and Hung and Thompson (1980), who concluded that iterative methods are more versatile and provide more data than analytical solutions, but are difficult and time-consuming to use (normally requiring a computer).

Cleland and Earle (1977) modified Plank's (1941) analytical equation and showed that their version gave as good agreement as iterative methods for plate freezing of slabs of Karlsruhe test substance (methylcellulose gel), minced beef and mashed potato.

A prerequisite of the method is an estimate of the overall heat transfer coefficient (h) for the combination of packaging and freezing system being used. Arce and Sweat (1980) provided a comprehensive survey of published heat transfer coefficients and methods of measurement encountered in food refrigeration processes. Other workers measured h in plate freezers using steady state (Templeton and Nicholson, 1972; Lorentzen, 1974) or transient temperature methods (Cowell and Namor, 1967), heat flux sensors (Gorbatov et al., 1977) or by simply calculating the value of h needed to give agreement between predicted and experimental freezing times (Cullwick and Earle, 1971). We found no data giving h for the types of packaging commonly used at present in the meat industry.

The aim of this work was: (1) to determine the value of h in the same plate freezer as previous experimental work (Creed and James, 1983) by using the transient temperature method which closely mimicked the practical situation of liver in contact with the freezer plate and the final product shape; (b) to use these data in a modification of Plank's equation (Cleland and Earle, 1977) to predict freezing times that could be compared to those found experimentally; (c) to determine values of h in the same plate freezer for packaging combinations found in normal commercial use; and (d) to use these data to predict the likely effect of the packaging on the freezing time of liver blocks.

MATERIALS & METHODS

Theory of the transient temperature method for the determination of the overall heat transfer coefficient

Consider a block of test material placed against the freezer plate. If the heat flow through the surface of the block to the plate is assumed to be one-dimensional with a direction perpendicular to the plate, it can be equated to the change in energy of the block, assuming no temperature difference throughout the block at any time, i.e.

$$hA(T_b - T_a) = Mc_p \frac{dT}{dt} \tag{1}$$

or

$$\frac{hAdt}{Mc_p} = \frac{dT}{(T_b - T_a)} \tag{2}$$

by integration (2) becomes:

$$\frac{hAt}{Mc_p} = Log_e(T_b - T_a) \tag{3}$$

or

$$t = \frac{Mc_p}{hA} Log_e(T_b - T_a) \tag{4}$$

A plot of time, t, against $Log_e(T_b - T_a)$ will give the slope

$$m = \frac{Mc_p}{hA} \tag{5}$$

from which the overall heat transfer coefficient, h, can be calculated by:

$$h = \frac{Mc_p}{Am} \tag{6}$$

Cowell and Namor (1967) concluded that, providing the Biot number was less than 0.1, the temperature throughout the object would be essentially uniform as assumed above, producing an error in h no greater than 3.2%. This constraint dictates that the thermal conductivity of the test object must be high.

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Practical determination of the heat transfer coefficient

A block of pure copper weighing 3.48 kg and measuring 0.034 by 0.075 by 0.15m was drilled and two copper-constantan thermocouples (twisted-pair PTFE-coated 0.2 mm diameter conductor) positioned just below the 0.075 by 0.15m face. The copper block was then embedded in a slab of expanded polyurethane foam 0.135 by 0.44 by 0.55m so that the largest face of the block was exposed on the largest face of the slab. Heat sink compound (white zinc oxide filled silicone) was applied to the surface of the block to simulate the pliable surface of liver thereby giving similar thermal contact. The packaging was then laid over the face of the block, care being taken that when polyethylene film was used, no creases or wrinkles were present. Details of the different packaging combinations used are shown in Table 1. Surface 'snow' caused by the condensation of atmospheric moisture was removed from the precooled freezer plate before the block was placed in contact at an applied hydraulic pressure of 310 kPa produced by a mid-setting on the freezer's hydraulic system. The temperature of the trichloroethylene entering the plates was controlled at $-40°C$ to $± 0.5°C$. The aluminum freezer plates measured 1.14 by 0.52 by 0.021m. Further details of the plate freezer used are given by Creed and James (1983).

Measurements

Refrigerant temperatures were monitored on the flow and return pipes from the freezer plate using copper-constantan thermocouples inserted into thermowells welded into the pipework. These thermocouples and those in the copper block were connected to a datalogging system resolving to $0.1°C$ and accurate to $± 0.5°C$. Data was recorded every 20 or 60 seconds during each run, the recording interval determined by the rate of temperature fall for the situation under study.

Prediction of freezing times

The modification of Plank's equation by Cleland and Earle (1977) used to calculate freezing times shown as Eq. (7) to (9), assumes that the latent heat of freezing is removed at T_f and that the thermal properties of the frozen material have a constant value.

$$Z = \frac{\rho \Delta H}{(T_f - T_a)} \left(\frac{PD}{h} + \frac{RD^2}{k_s} \right) \tag{7}$$

where

$$P = 0.5072 + 0.2018 Pk + Ste \left(0.3224 Pk + \frac{0.0105}{Bi} + 0.0681 \right) \tag{8}$$

and

$$R = 0.1684 + Ste(0.2740 Pk - 0.0135). \tag{9}$$

These equations were used by substituting the value of h determined experimentally, the previous experimental data for D and T_a (Creed and James, 1983) and thermal properties described below to obtain the values of the Biot, Plank and Stefan numbers in Eq. (8) and (9) and hence the factors P and R for use in Eq. (7).

No published data on the thermal properties of pig liver were located. Data for c_{pl} (3.37×10^3 J/kg°C from Robinson, 1972), ΔH (175×10^3 J/kg from Latyshev, 1978) and ρ (1057 kg/m³ from Poppendiek et al., 1966) for beef liver together with calculated values of k_s (1.14 W/m°C) and c_{ps} (1.72×10^3 J/kg°C) from the formulae of Sweat (1975) and Siebel (1892) assuming a mass fraction of water in pig liver of 0.695 (Paul and Southgate, 1978), were therefore used in Eq. (7) to (9). k_s was calculated assuming an average liver temperature of $-20°C$ as the freezing time was defined as the time for the center to reach $-7°C$, by which time the surface would be between $-40°$ and $-20°C$.

RESULTS

Determination of the overall heat transfer coefficients

The heat transfer coefficients were calculated using a computer program based on the method described earlier, with T_a taken as the mean of the inlet and outlet refrigerant temperatures. The results are shown in Table 2 for the different heat transfer situations studied.

Combination 3 corresponds closely to the experimental situation for freezing liver previously reported (Creed and James, 1983) and produced an h value of 361 W/m²°C. The

addition of a layer of solid fibreboard (1.5 mm thick) reduced this value by a factor of almost 8 and by over 32 when two layers of corrugated board (each layer 2.8 mm thick) were added to the polyethylene film. The Biot numbers obtained for each combination were all well below the upper limit of 0.1.

The reciprocal of the overall heat transfer coefficient (the thermal resistance) between the refrigerant and the copper block can be defined as the sum of the resistances of and between each layer. Values of 9.55×10^{-5} and 1.87×10^{-5} m²°C/W for the thermal resistance of the 30 micron polyethylene film and the 4.5 mm thick aluminum freezer plate respectively, were calculated by dividing the thermal conductivity of the material by its thickness. The values of h for each combination were then used together with the above resistances to produce a set of simultaneous equations whose solution provided the thermal resistances of each layer (Table 3).

Comparison of experimental and predicted freezing times

A value of 361 W/m²°C was substituted into Eq. (7) to (9) to obtain predicted freezing times covering the experimental range of conditions. These times were compared with previous experimental data (Creed and James, (1983). Fig. 1 shows the differences between predicted and experimental freezing time plotted against predicted freezing time. A least-squares curve-fitting procedure gave the best fit as a linear relationship of the form:

$$Z_p - Z_e = 0.39513 - 0.116023.Z_p \tag{10}$$

The correlation coefficient of this expression was 0.620.

The average absolute percentage error for 44 results was 6.50% (standard deviation 6.00) with a range from -12.0 to $+32.7\%$. If 2 experimental outliers were excluded, the average was 5.45% (standard deviation 3.47) with a range from -12.0 to $+12.8\%$.

Predicted freezing times for other heat transfer coefficients

Eq. (7) to (9) were used to produce the relationship shown in Fig. 2 between h and predicted freezing time for 7.6 and 15.2 cm blocks initially at $4°$ or $24°C$ and frozen at $-20°$ or $-40°C$.

DISCUSSION

THE MAJOR ADVANTAGES of plate freezing stem from the higher rates of heat transfer that can be obtained compared to air blast freezing systems. For a particular plate freezer the value of the overall heat transfer coefficient will depend upon the material and construction of the plate and the flow rate and type of refrigerant used.

Table 1—*Combinations of packaging used for determination of overall heat transfer coefficients*[a]

Combination	Layers between refrigerant and copper block
1	R : P : B
2	R : P : HSC : B
3	R : P : POLY : HSC : B
4	R : P : 2 X POLY : HSC : B
5	R : P : HSC : POLY : HSC : B
6	R : P : SOL : HSC : B
7	R : P : 2 X SOL : HSC : B
8	R : P : CORR : POLY : HSC : B
9	R : P : 2 X CORR : POLY : HSC : B

[a] R = Refrigerant; P = Plate; HSC = Heat sink compound; POLY = Polyethylene film 30 micron; SOL = Solid fibreboard, polyethylene-coated, 1.5 mm thick; CORR = Corrugated fibreboard, B-flute, 2.8 mm thick; B = Copper test block.

Table 2—Mean values of heat transfer coefficient in a vertical plate freezer for various combinations of packaging[a]

Combination	Number (Table 1)	Mean value W/m²°C	Standard deviation as % mean	Number samples	Biot Number
No wrapping	1	481[a][a]	14.8	8	0.04
No wrapping (+ HSC)	2	878[b]	15.0	10	0.08
Polyethylene (1 layer)	3	361[c]	14.7	10	0.03
Polyethylene (2 layers)	4	278[c]	25.6	7	0.02
Polyethylene (1 layer + HSC)	5	463[a]	16.7	7	0.04

[a] Above means with different subscripts are significantly different (P < 0.05)

Solid fibreboard	6	47[d][b]	5.8	10	0.004
Solid fibreboard (2 layers)	7	26[e]	2.2	7	0.002
Corrugated fibreboard (+ polyethylene)	8	20[f]	7.5	11	0.002
Corrugated fibreboard (2 layers + polyethylene)	9	11[g]	4.8	7	0.001

[b] Above means with different subscripts are significantly different (P < 0.001)
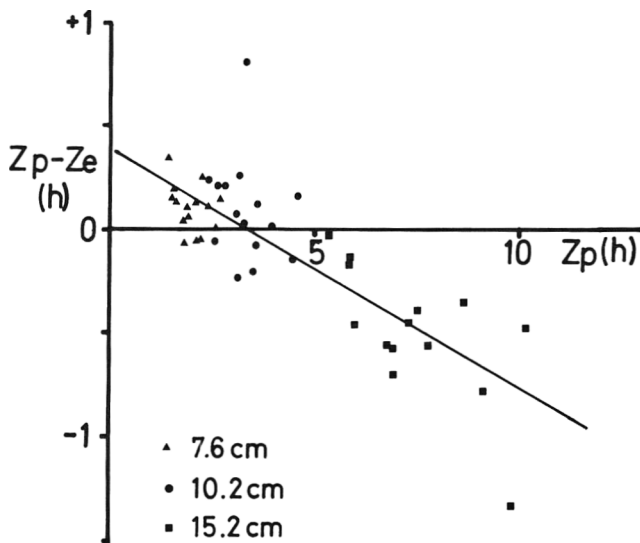
Fig. 1—Relationship of difference between predicted and experimental freezing times $(Z_p - Z_e)$ with predicted freezing times (Z_p), showing regression line (Eq. 10).

Fig. 2—Relationship between overall heat transfer coefficient (h) and predicted freezing times (Z_p) of liver blocks 7.6 or 15.2 cm thick frozen at −20° or −40°C from 4° or 24°C to −7°C at the center.

In the present work with good thermal contact between the measurement system and the plate, an average value of 878 W/m²°C was obtained. Cowell and Namor (1867) reported a value of 681 W/m²°C using the same method of measurement, but with an ethylene glycol/water refrigerant, while Templeton and Nicholson (1972) predicted a value of 430 W/m²°C for trichloroethylene at the flow rate used in these experiments. Although these values of h, for freezing in good contact vary by a factor of over 2, differences of this order in the range between 400 and 1000 W/m²°C have a small effect on freezing time (Fig. 2).

The addition of a single layer of polyethylene wrapping, which mimicked the experimental situation with liver, reduced the average value of h to 361 W/m²°C. When this value was used in Eq. 7, there was good agreement between the predicted and experimental freezing times. However the correlation shown in Fig. 1 indicates that there is a systematic linear relationship between freezing time and the error in prediction, over-estimates being made at freezing times of less than 3 hr and under-estimates above 3 hr.

An explanation of this effect could be that the thermal properties in all high water content foodstuffs are not constants as assumed in Eq. (7) to (9) but vary with temperature: the thermal conductivity of meat increasing and the specific heat decreasing as the temperature is progressively lowered below the initial freezing point. The average temperature at the end of the freezing operation of a thin block with consequently a short freezing time, is lower than that of a thick block with a long freezing time. Therefore the value of k_s would be greater than that calculated for use in the prediction equation. If a greater value of k_s was used in the prediction the second term in Eq. 7, RD^2/k_s would decrease. Similarly the value of c_{ps} would be smaller, decreasing Ste and hence the factors P and R. These two effects would therefore decrease the freezing time estimate, bringing it closer to the experimental value. By the same argument, for thick blocks with longer freezing times and a higher final average temperature, a smaller value of k_s and a greater value of c_{ps} than those used, would estimate a longer freezing time closer to the experimental results. For example, for run 32 (experimental freezing time 9.88 h for 15.2 cm block, (Creed and James, 1983), a freezing time of 9.1 h is predicted using the thermal properties previously mentioned evaluated for a final average temperature of −20°C. If a lower value of k_s, 1.06 W/m°C (evaluated at −10°C) and a higher value of c_{ps}, 2 × 10³ J/kg are used, a much closer estimate, 9.8 h is obtained. Cleland and Earle (1982) used a numerical technique that accounted for the variation of thermal properties with temperature but still found a comparable error curve. They suggested that the thermal properties were a function of freezing rate. Further work is required to clarify this situation. In practice, Eq.

(7) is accurate enough (within ± 12%) for most design applications.

Transporting and storing plate-frozen blocks of fish or raw material for pet food unwrapped is common practice, but long term frozen storage of unwrapped offal blocks results in substantial weight loss and surface desiccation and is not to be recommended. Wrapping before freezing would stop this deterioration and overcome one of the major disadvantages of plate freezing — double handling due to the practice of packing the product after rather than before freezing (Herbert and Visser, 1982). Freezing liver inside a polyethylene liner would seem to present little difficulty and the resulting flat blocks should stack easily on pallets for storage and transport. This investigation has shown that a single layer of polyethylene film reduces h by a factor of over 2 but does not substantially increase the freezing time, while a second layer further reduces h to 278 W/m$^{2\circ}$C. From Fig. 2 two layers would produce a maximum increase of only 8% in freezing time compared with no wrapping.

In a survey of commercial meat freezing operations, the two main wrapping combinations were solid fibreboard cartons with a bonded polyethylene inner layer, and corrugated fibreboard cartons with separate polyethylene film liners (Creed and James, 1981). Measurements carried out in this investigation produced average h values of 47 and 20 W/m$^{2\circ}$C respectively for these two combinations. Over the range of conditions shown in Fig. 2 (7.6-15.2 cm thick blocks. refrigerant temperatures −40° to −20°C. initial liver temperatures 4-24°C), freezing times compared with unwrapped blocks would be increased by between 1.2 and 5.5 hr (100-240%) for the first wrapping combination and 2.9 and 13.6 hr (50-123%) for the second. In previous investigations the average value of h for freezing meat blocks packed in polyethylene lined corrugated cartons in an air blast freezer (5 m/s), was found to be approximately 10 W/m$^{2\circ}$C (James et al., 1979). It is therefore clear from Fig. 2 that freezing times in a plate freezer would still be substantially shorter than in an air-blast system.

The thermal resistance determined for the solid fibreboard, 0.017 m$^{2\circ}$C/W (Table 3), was comparable to the value 0.024 m$^{2\circ}$C/W given by MacFarlane (1963). In contrast the value for corrugated board, 0.040 m$^{2\circ}$C/W (Table 3) was lower than that given by Mawson and Collinson (1977), 0.072 m$^{2\circ}$C/W for a similar material due to the effect of the freezer plate compressing the fibreboard.

Although plate freezing is widely used in the fish and pet food industries because of the advantages over conventional air-blast freezing of shorter freezing times and hence higher throughput, the method has yet to be taken up to any large extent in the U.K. meat industry. This investigation has provided data on overall heat transfer coefficients for typical commercial packaging combinations, and shown that a relatively simple prediction method can give results accurate enough for most design engineers. This should enable them to better appreciate the potential of this method of freezing foodstuffs.

LIST OF SYMBOLS

A = Area of contact between block and freezer plate (m^2)
Bi = Biot number (hD/k) (dimensionless).
c_p = Specific heat (J/kg$^\circ$C)
D = Thickness of food material (m)
h = Overall heat transfer coefficient (W/m$^{2\circ}$C)
k = Thermal conductivity (W/m$^\circ$C)
L = Latent heat (J/kg)
M = Mass of block (kg)
m = Slope of plotted straight line (s)
P = Shape factor in Plank's equation (dimensionless).
Pk = Plank number ([c_{pl} (T$_i$ − T$_f$)]/ΔH) (dimensionless).
R = Shape factor in Plank's equation (dimensionless).
Ste = Stefan number ([c_{ps} (T$_f$ − T$_a$)]/ΔH) (dimensionless).
T = Temperature ($^\circ$C)
T$_a$ = Temperature of the refrigerating medium ($^\circ$C)
T$_b$ = Temperature of the block ($^\circ$C)
T$_f$ = Initial freezing point of material ($^\circ$C)
T$_i$ = Initial temperature ($^\circ$C)
t = Time (s)
Z = Freezing time (s)
Z$_e$ = Experimental freezing time (h)
Z$_p$ = Predicted freezing time (h)

Greek symbols

ρ = Density (kg/m^3)
ΔH = Total enthalpy change between T$_f$ and −7°C. (J/kg).

Subscripts

l   Relating to unfrozen material.
s   Relating to frozen material.

REFERENCES

Arce, J.A. and Sweat, V.E. 1979. Survey of published heat transfer coefficients encountered in food refrigeration processes. ASHRAE (American Society of Heating, Refrigerating and Air-Conditioning Engineers) Project RP-228.
Bakal, A. and Hayakawa, K-I. 1973. Heat transfer during freezing and thawing foods. Adv. Food Res. 13: 217.
Cleland, A.C. and Earle, R.L. 1977. A comparison of analytical and numerical methods of predicting the freezing times of foods. J. Food Sci. 42: 1390.
Cleland, A.C., Earle, R.L., and Cleland, D.J. 1982. The effect of freezing rate on the accuracy of numerical freezing calculations. Int. J. Refrig. 5: 294.
Cowell, N.D. and Namor, M.S.S. 1967. Heat transfer coefficients in plate freezing: the effect of packaging materials. Annexe 1974-3 Bull. Int. Inst. Refrig. 45.
Creed, P.G. and James, S.J. 1981. A survey of commercial meat block freezing in the United Kingdom and Eire. Int. J. Refrig. 4: 348.
Creed, P.G. and James, S.J. 1983. The freezing times of liver in a vertical plate freezer. Proc. 16th Int. Congress Refrig., Paris.
Cullwick, T.D.C. and Earle, R.L. 1971. Prediction of freezing times of meat in plate freezers. Proc. 13th Int. Congress Refrig., Washington, USA., 3: 397.
Gorbatov, V.M., Ionov, A.G., Khromov, V.I., and Mekenitsky, S.Y. 1977. Problems of meat products freezing in blocks. Proc. 23rd European Meeting of Meat Research Workers., Moscow, USSR, Paper D16.
Herbert, L.S. and Visser, K. 1982. A large capacity plate freezer for meat cartons. Annexe 1982-1 Bull. Int. Refrig. 107.
Hung, Y-C, and Thompson, D.R. 1980. Freezing time predictions — A review. Paper No. 80-6501 presented at 1980 Winter Meeting of the American Society of Agricultural Engineers, Chicago, IL, Dec. 2-5.
James, S.J., Creed, P.G., and Bailey, C. 1979. The determination of the freezing time of boxed meat blocks. Proc. Inst. Refrig. 75: 74.
Kinder, E. and Lamb, J. 1974. The prediction of freezing times of foodstuffs. Proc. 3rd Meat Research Institute Symp. "Meat Freezing — Why and How." Meat Research Institute, Langford, Bristol, U.K., 17.1.

Table 3—Thermal resistances and conductances calculated from data in Table 2

Layer or interface	Thermal resistance m$^{2\circ}$C/W	Thermal conductance W/m$^{2\circ}$C
Solid fibreboard	0.017	58.0
Corrugated fibreboard	0.040	25.2
Heat sink compound	0.00093	1081.3
Refrigerant/Plate interface	0.00020	
Plate/block interface	0.0019	
Plate/polyethylene interface	0.0015	
Polyethylene/ Polyethylene interface	0.0007	
Plate/solid fibreboard interface	0.0030	
Plate/corrugated fibreboard interface	0.0095	

Damage to Pork Liver Caused by Repeated Freeze-Thaw Cycling and Refrigerated Storage

ELIZABETH D. STRANGE, SUSAN B. JONES, and ROBERT C. BENEDICT

──────── ABSTRACT ────────

The effects of freezing and thawing (F-T) and refrigerated storage (R-S) on pork liver were examined. Intact cells were isolated from the tissue and analyzed for membrane integrity and the tissue extracts were analyzed for protein content. Significant ($p < 0.05$) differences were noted in the number of isolated intact cells and in tissue protein content between F-T and R-S or fresh livers. Differences in cell membrane integrity were also noted between fresh and R-S livers. Repeated freezing and thawing appears to break down the liver structure differently than does refrigerated storage.

INTRODUCTION

IN 1978 the United States exported approximately $205 million worth of variety meats but experienced rejection rates of 5-30% at port of entry as "unfit for human consumption" or for other reasons (Turczyn, 1980). Miller and Bongers (1981) stated that blood leakage or stains on boxes indicated that the product had been defrosted after initial freezing. Livers were the variety meat most often rejected (Private Communication, T. H. Camp).

Although important economically, the deterioration of liver during storage, freezing, and thawing has not been examined extensively. Smith et al. (1983a) reported that freezing temperature had little effect on weight loss, color score, overall appearance, odor, and tenderness of beef livers. Smith et al. (1983b) also indicated that vacuum packaging as soon as possible after slaughter gave the most acceptable appearance to chilled beef liver destined for retail display.

Two recent studies (Berry et al., 1982; Hanna et al., 1982) showed that frozen storage had no significant effect on the total microbial populations (aerobic plate counts) of stored variety meats. The study by Berry et al. (1982) included transportation at above freezing temperatures following freezing. Hamm and Masic (1975) reported on a method to distinguish fresh from frozen liver, and Partmann (1973) examined the histological effects of different freezing and thawing rates. Heinz (1974) contrasted frozen-stored liver with fresh liver, and reported that freezing had no adverse effects on the use of liver in products, but that freezing and thawing increased the amount of drip. Smith et al. (1983a) found that uncut frozen beef livers had less drip than sliced frozen liver. Smith et al. (1983b), however, showed that frozen and thawed liver had less weight loss than did chilled liver.

The objective of this study was to measure the damage to liver tissue and to elucidate patterns of degradation during refrigerated storage and after repeated freezing and thawing.

MATERIALS & METHODS

WHOLE PORK LIVERS were obtained from a local slaughterhouse immediately following Federal inspection and removal of the bile

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duct and gall bladder. Each liver was divided into seven samples (approximately 125-150g). The samples were vacuum packaged and sealed with a Smith SuperVac in 7 X 7 sized "IKD ALL-VAC 13 FBR" pouches. Temperature was monitored by insering a YSI temperature probe into the center of a tissue sample and continuously recording temperatures. Samples were stored at either $-20°C$ or $+5°C$ within 3 hr of slaughter. The sample from each liver which was analyzed fresh was not vacuum packaged. A total of six whole pork livers were used: three for freeze-thaw cycling (F-T) and three for refrigerated storage (R-S).

Freeze-thaw cycled liver samples were initially frozen for 70 hr at $-20°C$ in a freezer-incubator. The freezer-incubator was then programmed to hold the temperature at $+5°C$ for 24 hr, followed by $-20°C$ for 24 hr. Each 48 hr freezing-refrigerated storage period was designated as one freeze-thaw cycle. This cycling was continued for 11 days for a total of six cycles. Liver samples were analyzed at the end of the thaw portion of each cycle.

The refrigerated storage liver samples were placed in the freezer-incubator set at $+5°C$. Refrigerated storage of 24 hr was called a cycle. Frozen-thawed and refrigerated storage samples with the same cycle number have equivalent time in refrigerated storage.

Livers were monitored to assess drip formation, number of isolated cells and their viability, and protein content of the tissue extracts. Analysis was carried out on day of slaughter (fresh) (except for drip) and after 1, 2, 4, 5, and 6 F-T or R-S cycles.

The amount of drip formed at each cycle was determined by weighing the drained liver sample and measuring the amount of liquid left in the package. Percentage drip was reported as (volume liquid (mL)/weight drained sample (g) + weight of liquid (g)) X 100.

Phosphate buffered saline (PBS) contained 0.2g KCl, 0.2g KH_2PO_4, 2.89g $Na_2HPO_4 \cdot 12H_2O$, and 7.0g NaCl per liter (pH 7.4). PBS + 0.5 mM EGTA (ethyleneglycol-bis-(-amino-ethyl ether) N,N'-tetraacetic acid) and 1% w/v Triton X100 in PBS were also prepared.

Modified magnesium-free Hanks balanced salt solution (HBSS) contained 0.4g KCl, 0.06g KH_2PO_4, 0.12g $Na_2HPO_4 \cdot 12H_2O$, 8.0g NaCl, 2.19g $NaHCO_3$ per liter (pH 7.4) (Moldeus et al., 1978). The 0.05% collagenase Type IV (Sigma) – 0.01% hyaluronidase Type II (Sigma) solution was prepared immediately before use in HBSS + 5 mM $CaCl_2 \cdot H_2O$.

A filtered 0.5% trypan blue solution in PBS was diluted with an equal volume of PBS immediately before use. A 200-μL aliquot of a 0.5% solution of fluorescein diacetate (FDA) in acetone was diluted with PBS to a final concentration of 0.5 μg/mL (Rotman and Papermaster, 1966).

Cell isolations were carried out in duplicate. The liver cell isolation procedure (Fig. 1) was adapted from that of Fry et al. (1976). Three grams of 0.5-mm thick liver slices (about 20 slices) were prepared with a Stadie-Riggs tissue slicer. As each slice was cut, it was placed into a tared 125-mL Erlenmeyer flask containing 10 mL PBS. Slicing took about 15 min per isolation. The sliced liver tissue was washed two times with 10 mL PBS for 10 min each time. The sample flasks were placed in a 37°C water bath and shaken at 100 RPM for tissue washings and enzymatic digestion. The PBS was decanted and labeled PBS. Washing was continued with two 10-mL portions PBS + 0.5 mM EGTA, a Ca^{+2} chelating buffer, for 10 min each, and this decantate was labeled PBS-EGTA. The washed liver slices were then digested for 60 min with 10 mL enzyme solution. After completion of the incubation, the cells were released from the liver tissue by gentle agitation with a stirring rod on a 100-mesh screen in a Cellector. The liver tissue in the Cellector was washed twice with 5-mL portions ice cold PBS. Tissue debris remaining on the screen was discarded. The filtrate contained the liver cells and cell debris. Cells were separated from the cell debris by centrifugation at 80 X g for 5 min and the pellets washed three times in 10-mL portions cold PBS for a total centrifugation time of 20 min. The supernate from

the pellets was saved and labeled CELL DEBRIS. The isolated cells were then suspended in PBS, and kept cold until counting and protein determinations were completed.

A Hausser Hy-Lite Ultra Plane hemocytometer was used for cell counting. Isolated cells were treated with FDA by adding 100 μL of an appropriate dilution of the cells to 9.9 mL FDA solution. The fluorescing cells were counted immediately using blue excitation illumination at 125X magnification. Fluorescing cells are assumed to have an intact cell membrane (Rotman and Papermaster, 1966). Cells visible by phase contrast microscopy also were counted in the same hemocytometer chamber at the same magnification.

Isolated cells were stained with trypan blue by adding 100 μL of an appropriate dilution of the cell suspension to 9.9 mL trypan solution. After staining for 1 min, cells were counted under bright field illumination and then under phase contrast. Viable cells with an intact cell membrane exclude trypan (Patterson, 1979). Phase counts were reported as number of cells X 10^7/3g liver tissue and the ratios of FDA count-to-phase count and of trypan count-to-phase count were calculated and reported. All round particles visible in the microscope field were counted.

Biuret protein determinations were performed on all extracts and isolated cell fractions. Isolated cell fractions were solubilized with 1% Triton X100 before biuret determination. A biuret protein standard (bovine serum albumin) was run daily.

For histologic examination, tissue samples were fixed in pH 7, 0.08M PO_4 buffered, 10% formalin before sectioning with a freezing microtome. The 20 micron sections were stained with hematoxylin and eosin, mounted, and photographed at 50X with a Nikon Optiphoto microscope.

Differences in means of parameters measured were evaluated by the "t" test described in Steel and Torrie (1980). Linear cycling effects were determined by analysis of variance using a general linear models procedure. Significance was tested at the p < 0.05 level.
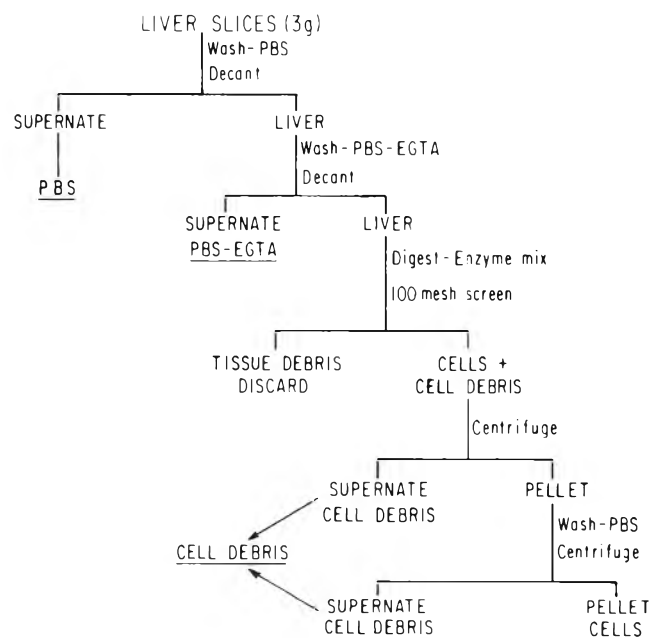
RESULTS & DISCUSSION

THE TEMPERATURE VS TIME curve for typical 48-hr freeze-thaw cycle is shown (Fig. 2). The time for sample freezing or thawing varied with sample size, and the slope of the line generated by monitoring the temperature of liver samples during freezing and/or thawing was not constant. The temperature/time curve flattened when the sample reached $-2°$C because of the phase change of liquid H_2O to ice. The time at $-2°$C was extended during the thawing portion because of a smaller temperature differential between sample and ambient temperatures. Hamm et al. (1982) reported that super-cooling effects were possible during the freezing of meat products and caused an increase in the overall rate of freezing. We found no evidence for this effect in the monitoring of our samples (Fig. 2).

Percent drip increased for both F-T and R-S livers and had a significant linear relationship with cycling (Table 1). While freeze-thaw cycling produced more drip than refrigerated storage, these differences were not significant. The differences between fresh and F-T liver, however, were significant at cycles 4, 5, and 6, and the differences between fresh liver and R-S liver were significant at cycle 1 (Table 1). Smith et al. (1983b) showed that frozen-thawed whole beef livers had less weight loss than did chilled livers. Holding time for chilled livers was 15.5 days, and the frozen-thawed livers were frozen only once. Weight loss from drip increased as a function of holding time and drip from F-T cycle 1 was less than drip from R-S cycle 6 (3.1% vs 5.7%).

The cell isolation procedure (Fig. 1) involved stepwise detachment of the cells from each other and from the collagen matrix which surrounds the cells. The PBS wash removed protein not tightly held within the liver structure. The PBS-EGTA wash disrupted the desmosomes, part of

Fig. 1—Flow diagram for cell isolation procedure. Fractions underlined were measured for protein content.
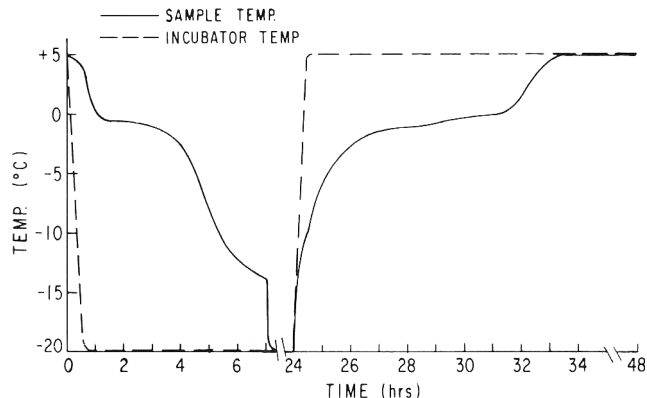
Fig. 2—Temperatures during a typical 48-hr freeze-thaw cycle.

Table 1—Mean percent drip and standard deviations for freeze-thaw (F-T) (N = 3) and refrigerator stored (R-S) (N = 3) livers

	Fresh % (S.D)	Cycle 1 % (S.D.)	Cycle 2 % (S.D.)	Cycle 4 % (S.D.)	Cycle 5 % (S.D.)	Cycle 6 % (S.D.)
F-T*[a]	0[b]	3.1 (2.1)	5.5 (3.6)	8.9 (2.4)*[c]	11.4 (5.3)*	10.5 (4.8)*
R-S*	0	1.9 (0.9)*[d]	2.7 (2.7)	3.4 (3.7)	5.9 (4.6)	5.7 (5.6)
		N.S.[e]	N.S.	N.S.	N.S.	N.S.

[a] * p < 0.05 for the F value from analysis of variance for the linear effect of cycle on percent drip.
[b] Percent drip for fresh liver defined as 0.
[c] * Probability of a larger value of t < 0.05 for differences in percent drip between fresh and F-T livers.
[d] * Probability of a larger value of t < 0.05 for differences in percent drip between fresh and R-S liver.
[e] N.S. — not significant — probability of a larger value of t > 0.05 for differences in drip between F-T and R-S livers.

the tissue structure involved in cell-to-cell adhesion (Seglen, 1979). The PBS-EGTA fraction contained protein not removed by the first PBS wash plus proteins which were solubilized or loosened by Ca^{+2} chelation. The enzymatic digestion loosened the collagen surrounding the cell and some intercellular junctions. Agitation on the Cellector screen destroyed the gap and tight junctions between the cells (Van De Werve, 1980). The cell debris contained matter which did not sediment at $80 \times g$, and the cell fraction contained the isolable cells in 3g liver. Cell protein was the protein in the isolable cell fraction and total protein was the protein recovered in the tissue fractionation scheme.

Tables 2 and 3 present data obtained from the cell isolation procedure performed on fresh, freeze-thaw (F-T) and refrigerator stored (R-S) livers. F-T fresh livers were significantly different from R-S fresh livers only in the phase count-number of cells isolated (Tables 2 and 3). Livers used in the R-S study yielded significantly higher numbers of cells because they contained considerably more red blood cells (for unknown reasons) in the isolated cell fraction than the livers used in the F-T study. These red blood cells were included in the counts since they were identifiable only by size with the magnification used, and nonparenchymal liver cells of a similar size exist (Homma et al., 1982).

The relationship between cell count and cell protein content is shown in Fig. 3. The livers used for the F-T studies had a higher protein content per cell than did the livers used for the R-S studies, indicating that the R-S livers contained larger numbers of smaller cells.

The number of isolated cells (phase-count) showed a significant linear decrease as storage time or cycle number increased for both F-T and R-S livers. The differences

between the number of cells isolated from fresh liver and F-T liver, and from fresh liver and R-S liver were significant after 4, 5, and 6 cycles. The number of cells isolated from F-T livers were always significantly smaller than the number isolated from R-S livers (Table 2). Because the R-S group of livers had higher cell counts when fresh, the import of the differences between F-T and R-S is unclear.

To compare effects of the different storage regimes on the isolability of the liver cell, the relative yield of isolable cells was calculated as a ratio of number of cells obtained from stored livers to number of cells obtained from fresh livers (Table 2).

Relative yields of the isolable cells showed significant differences for all three types of comparisons. F-T livers had significantly smaller ratios than fresh liver by cycle 2, but the R-S livers showed no significant differences until cycle 5. The relative yield of isolable cells from F-T liver was always less than from R-S liver and was significantly different for cycles 4 and 5.

Cell protein showed a significant linear decrease as cycle number increased for both F-T and R-S livers (Table 3). Cell protein of F-T liver was significantly lower than fresh liver and R-S liver for all cycles. However, cell protein of R-S liver was significantly smaller than fresh liver at cycle 6 only (Table 3).

Differences in phase count, relative yield ratios, and cell protein indicated that freezing and thawing destroy the liver cell much more rapidly and to a greater extent than refrigerated storage. Each freezing and thawing cycle destroyed 20% of the remaining isolable liver cells so that after six freezing and thawing cycles only 25% of the liver cells remain intact. However, the increase in the amount of drip formed during freezing and thawing was much less

Table 2—Means and standard deviations of parameters measured on isolated cells for freeze-thaw (F-T) (N = 3) and refrigerator stored (R-S) (N = 3) livers

	Fresh		Cycle 1		Cycle 2		Cycle 4		Cycle 5		Cycle 6	
Phase count — number (#) of cells isolated $\times 10^7$/3g of liver tissue												
	#	(S.D.)	#	(S.D.)	#	(S.D.)	#	(S.D.)	#	(S.D.)	#	(S.D.)
F-T*[a]	32.5	(22.3)	25.2	(16.5)	19.8	(12.7)	9.3	(2.5)*[b]	6.4	(1.4)*	7.8	(4.0)*
R-S*	129.2	(27.4)	127.7	(12.5)	104.2	(12.6)	99.9	(14.9)*[c]	81.1	(25.0)*	62.8	(13.6)*
		*[d]		*		*		*		*		*
Relative yield of isolable cells[e]												
	Ratio	(S.D.)	Ratio	(S.D.)	Ratio	(S.D.)	Ratio	(S.D.)	Ratio	(S.D.)	Ratio	(S.D.)
F-T[f]	1.00	(0)	0.814	(0.619)	0.623	(0.214)*[b]	0.394	(0.204)*	0.295	(0.190)*	0.267	(0.064)*
R-S[f]	1.00	(0)	1.031	(0.276)	0.844	(0.244)	0.793	(0.145)	0.617	(0.066)*[c]	0.511	(0.153)*
		N.S.[g]		N.S.		N.S.		*[d]		*		N.S.
FDA/phase counts ratios[h]												
F-T	0.542	(0.272)	0.615	(0.295)	0.576	(0.147)	0.677	(0.155)	0.716	(0.145)	0.665	(0.220)
R-S	0.638	(0.120)	0.672	(0.071)	0.612	(0.073)	0.548	(0.123)	0.492	(0.111)	0.610	(0.146)
		N.S.[g]		N.S.		*[d]		NS		*		N.S.
Trypan/phase counts ratios[i]												
F-T*[a]	0.676	(0.098)	0.686	(0.087)	0.732	(0.089)	0.816	(0.103)*[b]	0.886	(0.034)*	0.837	(0.134)*
R-S*[a]	0.592	(0.085)	0.766	(0.067)*[c]	0.771	(0.048)*	0.729	(0.045)*	0.741	(0.016)*	0.738	(0.049)*
		N.S.[g]		N.S.		N.S.		NS		*[d]		N.S.

[a] * p < 0.05 for the F value from analysis of variance for the linear effect of cycle on parameter measured.
[b] * Probability of a larger value of t < 0.05 for the differences in parameter measured between fresh and F-T livers.
[c] * Probability of a larger value of t < 0.05 for the differences in parameter measured between fresh and R-S livers.
[d] * Probability of a larger value of t < 0.05 for the differences in parameter measured between F-T and R-S livers.
[e] Ratio of the number of cells isolated at the specified cycle to the number of cells isolated when the same liver was fresh.
[f] Analysis of variance for linear effect not done.
[g] N.S. — not significant — probability of a larger value of t > 0.05 for the differences in parameter measured between F-T and R-S livers.
[h] Ratio of the number of cells visible when viewed with fluorescent illumination and stained with FDA to the number of cells visible under phase contrast illumination.
[i] Ratio of the number of cells visible when viewed under bright field illumination and stained with Trypan Blue to the number of cells visible under phase contrast illumination.

Table 3—Means and standard deviations of protein content of isolated cells and of tissue extracts for freeze-thaw (F-T) (N = 3) and refrigerator stored (R-S) (N = 3) livers

	Fresh mg (S.D.)	Cycle 1 mg (S.D.)	Cycle 2 mg (S.D.)	Cycle 4 mg (S.D.)	Cycle 5 mg (S.D.)	Cycle 6 mg (S.D.)
Cell protein[a]						
F-T*[b]	177 (51)	97 (16)*[c]	75 (45)*	37 (8)*	34 (3)*	38 (14)*
R-S*[b]	246 (93)	266 (72)	260 (69)	193 (20)	207 (97)	157 (19)*[d]
	N.S.[e]	*[f]	*	*	*	*
PBS protein[a]						
F-T	228 (8)	357 (75)*[c]	309 (46)*	275 (37)*	271 (37)*	233 (40)
R-S*[b]	233 (28)	240 (25)	245 (24)	288 (65)*[d]	286 (17)*	358 (50)*
	N.S.[e]	*[f]	*	N.S.	N.S.	*
PBS-EGTA protein[a]						
F-T*[b]	50 (2)	89 (4)*[c]	93 (10)*	82 (6)*	99 (9)*	110 (2)*
R-S*[b]	44 (7)	60 (10)	45 (8)	79 (20)*[d]	72 (5)*	75 (9)*
	N.S.[e]	*[f]	*	N.S.	*	*
Cell debris protein[a]						
F-T*[b]	284 (44)	280 (10)	258 (54)	226 (34)*[c]	214 (4)*	205 (15)*
R-S	221 (76)	216 (47)	187 (149)	181 (68)	172 (65)	190 (84)
	N.S.[e]	N.S.	N.S.	N.S.	N.S.	N.S.
Total protein[g]						
F-T[h]	713 (33)	809 (31)*[c]	722 (37)	606 (22)*	604 (18)*	572 (19)*
R-S[h]	730 (55)	767 (40)	724 (74)	726 (42)	723 (55)	766 (42)
	N.S.[e]	N.S.	N.S.	*[f]	*	*

[a] Protein measured by biuret reaction.
[b]* $p < 0.05$ for the F value from analysis of variance for the linear effect of cycle on parameter measured.
[c]* Probability of a larger value of t < 0.05 for the differences in parameter measured between fresh and F-T livers.
[d]* Probability of a larger value of t < 0.05 for the differences in parameter measured between fresh and R-S livers.
[e] N.S. — not significant — probability of a larger value of t > 0.05 for the differences in parameter measured between F-T and R-S livers.
[f]* Probability of a larger value of t < 0.05 for the differences in parameter measured between F-T and R-S livers.
[g] Calculated by summing the protein measurements made on the individual fractions that were isolated. Standard deviation determined by taking the square root of the sum of the variances and dividing by the number of fractions.
[h] Analysis of variance for linear effect not done.

than would be expected if drip contained most of the exudate from the destroyed cells. Both storage regimes eventually destroyed the liver cell to the extent that it could no longer be isolated by enzymatic techniques.

Viability of the isolated liver cells was estimated by two methods: FDA/phase count ratio and trypan/phase count ratio. Both methods indicated that approximately 50% of the isolated cells from fresh liver were viable.

This viability was lower than that reported by Fry et al. (1976) because of autolytic damage which begins at slaughter. The time elapsed from slaughter to viability measurements in Fry's study was about 2 hr, while in this study it was at least 6 hr.

As the proportion of viable cells decreases the FDA/phase count ratio should decrease and the trypan/phase count ratio should increase. However, the FDA/phase count ratios varied randomly with cycle number, and ratios differed significantly in only 2 of the 16 comparisons tested (Table 2). This lack of significant findings on changes in the viability of the isolated cells as measured by the uptake in FDA may result because as the number of fluorescein-containing (viable) cells decreased, the naturally fluorescent cells became more visible. Both populations of cells were counted as viable, causing random variations in FDA/phase count ratios.

Viability of the cells did decrease during F-T and R-S storage. The trypan/phase count ratio showed a significant linear increase as cycle number increased for both F-T and R-S livers (Table 2). F-T trypan/phase count ratios are significantly higher than fresh at cycles 4, 5, and 6 and R-S trypan/phase count ratios are significantly higher at all cycles (Table 2). The only significant difference between F-T and R-S trypan/phase count ratios was at 5 cycles.

R-S and F-T storage affected the viability of isolable liver cells differently. F-T treatment, while destroying some cells, preserved cellular functions related to trypan blue exclusion in others during the first two F-T cycles. By cycle 4, viability of surviving cells was significantly decreased. R-S storage, however, while not destroying the cells, caused significant decreases in viability after one cycle presumably from autolytic damage.

Increases in the amount of protein released by the PBS washes indicated a degree of structural damage to liver tissue where proteinaceous material was retained in the tissue sampled, but was easily removed by washing with PBS. The PBS protein fraction did not vary with cycle number in a linear manner for F-T livers but did for R-S livers (Table 3). F-T liver had significantly higher amounts of easily removed protein than fresh liver for all cycles but cycle 6. Damage caused by one freeze-thaw cycle was demonstrated by the increase in the amount of PBS protein isolated at one cycle. The decreasing amounts of PBS protein during extended F-T cycling indicated changes in the tissue sampled.

Damage to the liver tissue, as shown by PBS protein changes, during R-S followed a different pattern than during F-T. The increase in the PBS protein from R-S livers did not occur until cycle 4 and the maximum occurred at cycle 6. Maximum destruction of cells in R-S livers also occurred at cycle 6 suggesting that, for R-S livers, the material from the destroyed cells is removed in the PBS washes rather than contributing to the drip.

Damage to cell-cell adhesion mechanisms was demonstrated by significant linear increases in protein content of PBS-EGTA as the cycle number increased for both F-T and R-S livers. F-T livers had significantly higher amounts of
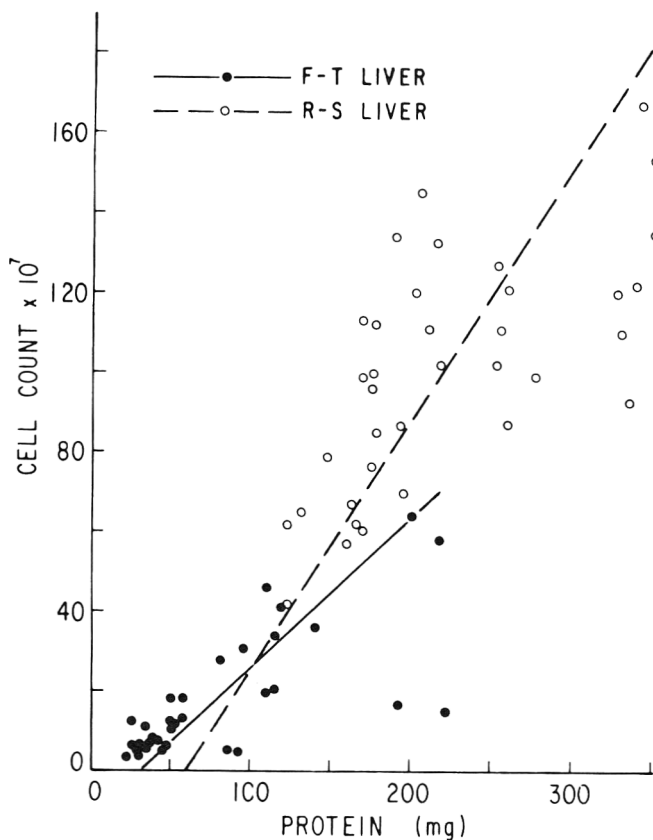
Fig. 3—Relationship between cell count and cell fraction protein content. Least squares fit for freeze-thaw liver data, r = 0.73. Least squares fit for refrigerated stored liver, r = 0.66.

Fig. 4—Hematoxlyin and eosin stained liver sections. A — Liver section, Fresh; B — Liver section, After six cycles of refrigerated storage; C — Liver section, After six freeze-thaw cycles; ◁ Collagen matrix; ◄■ Large spaces between cells not present in fresh liver.

protein extracted by PBS-EGTA than fresh liver throughout the storage, and R-S livers had significantly higher amounts of protein removed after cycles 4, 5, and 6 than fresh liver (Table 3). PBS-EGTA extracted significantly more protein from F-T than from R-S at cycles 1, 2, 5, and 6 (Table 3). Adhesion mechanisms were damaged either by the formation of intercellular ice and concomitant osmotic shrinkage of the cells in their supporting network of collagen, or by the mechanical stress of the intercellular ice. Partmann (1973) noted, in a histochemical study, that groups of liver cells were pressed apart by the formation of intercellular ice.

The amount of cell debris protein decreased significantly during cycling for F-T livers. However, the differences in cell debris protein between F-T and R-S livers, and fresh and R-S livers were not significant (Table 3).

The total protein recovered decreased during storage for F-T liver and remained about the same for R-S liver (Table 3). The total protein recovered in the isolation procedure was significantly lower for F-T liver than for R-S liver and for F-T liver than for fresh liver after 4, 5, and 6 cycles. Extensive damage to the cellular portion of the tissue during F-T caused an increase in the proportion of connective tissue, principally collagen (discarded as tissue debris in the cell isolation procedure), in the tissue sampled. An increase in the amount of a gelatinous substance extruded during tissue slicing was noted as the number of freeze-thaw cycles increased, and there was a gradual increase in the number of slices of tissue needed to obtain 3g of sample. This extensive destruction of cells, but not the collagen network which surrounds and supports the cells, may have an effect on the texture of the frozen liver. The effect of freezing on the texture of liver is not known. There were no significant differences when total protein recovered from fresh liver was compared with the total protein recovered from the R-S liver (Table 3).
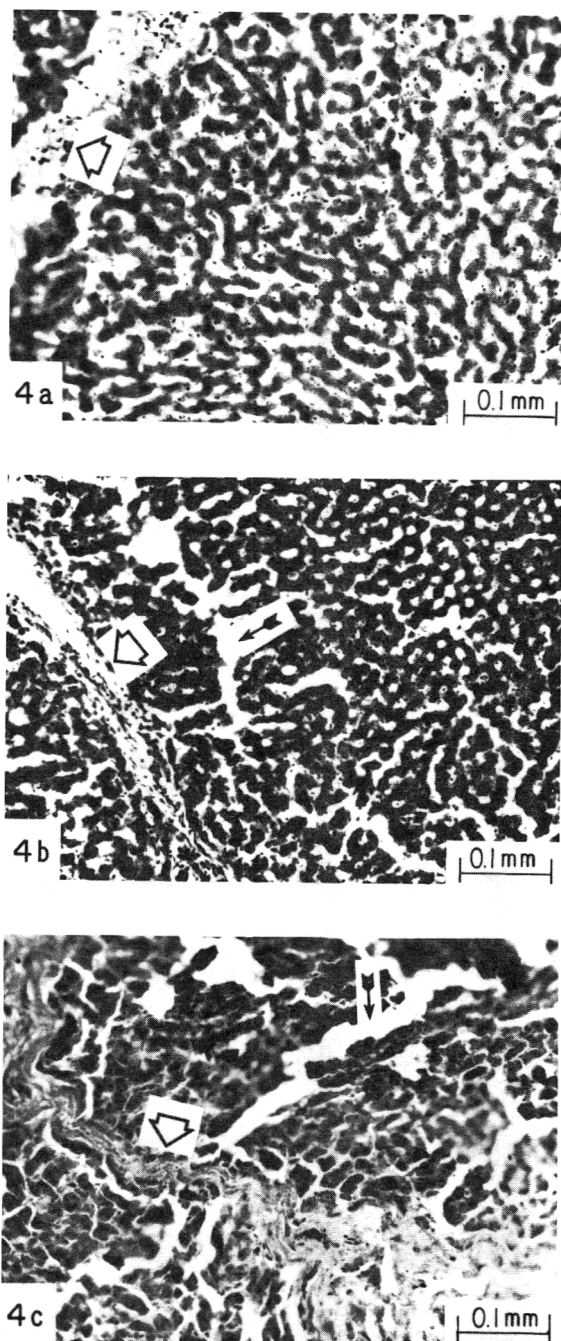
Fig. 4 shows the changes in the histology of the liver during storage. The collagen cage surrounding the lobule of the liver remained intact during both R-S and F-T. The liver cells in the R-S liver were partially destroyed and the spaces between the chords of the cells were enlarged. The micrograph of the F-T liver shows much more extensive damage to the cellular portion of the liver. Large areas of the lobule had no cells present, and the cells that are present had distorted nuclei. These micrographs confirm qualitatively some of the changes in the liver quantitatively measured in this study.

CONCLUSIONS

FREEZING AND THAWING liver tissue caused extensive damage to the liver cells but preserved some of the ability of surviving cells to exclude trypan blue during the first two cycles. Each freeze-thaw cycle destroyed about 20% of the remaining intact cells, but did not destroy the supporting collagen matrix. Damage to the cell-cell adhesion mechanisms was evident after one freeze-thaw cycle.

Damage to the liver tissue also occurred during refrigerated storage, but it was only after 4 days of storage that the rate of cell destruction matched that of F-T; damage to the cell-cell adhesion mechanisms was significant. Autolytic damage to the cells, as measured by viability, is a significant factor during refrigerated storage of liver.

REFERENCES

Berry, B.W., Rothenberg, C.A., Oblinger, J.L., Miller, W.R., Bonger, A.J. and Labots, H. 1982. Changes in the microbiological and shelf-life characteristics of beef tongues and livers following transcontinental and transoceanic shipment. Meat Sci. 7: 81.

Camp, T.H. 1983. Private communication. USDA-ARS, Meat Processing and Martketing Research, College Station, TX.

Fry, J.R., Jones, C.A., Wiebkin, P., Bellemann, P., and Bridges, J.W. 1976. The enzymic isolation of adult rat hepatocytes in a functional and viable state. Anal. Biochem. 71: 341.

Hamm, R., Gottesmann, P., and Kijowski, J. 1982. Einfrieren und Auftauen von Fleisch: Einflusse auf Muskelgewebe und Tausaftbildung. Die Fleischwirtschaft 62(8): 983.

Hamm, R. and Masic, D. 1975. Routinemethode zur Unterscheidung zwischen frischer Leber und aufgetauter Gefrierleber. Die Fleischwirtschaft 55(2): 242.

Hanna, M.O., Smith, G.C., Savell, J.W., McKeith, F.K., and Vanderzant, C. 1982. Microbial flora of livers, kidneys and hearts from beef, pork and lamb: effects of refrigeration, freezing and thawing. J. Food Prot. 45(1): 63.

Heinz, G. 1974. Qualitatserhaltung von Rinderleber unter Kuhloder Gefrierbedingungen. Die Fleischwirtschaft 54(4): 670.

Homma, S., Nagamori, S., Hasumura, S., Fujise, K., Sujino, H., Kameda, H., Kirino, Y., Hataba, Y., and Suzuki, T. 1982. The morphological studies on characteristics of isolated parenchymal and non-parenchymal cells of the rat liver. J. Electron Microsc. 31: 389.

Miller, W.R. and Bongers, A.J. 1981. Receivers' packaging preferences and packaging deterioration problems of U.S. variety meats observed at European markets. USDA, Advances in Agricultural Technology 6.

Moldeus, P., Hogberg, J. and Orrenius, S. 1978. Isolation and use of liver cells. Methods in Enzymology 52: 60.

Partmann, W. 1973. Histologische Veranderungen in Rind- und Schweinefleisch sowie Schweineleber unter definierten Gefrier- und Auftaubedingungen. Die Fleischwirtshaft 53(1): 65.

Patterson, M.K. 1979. Measurement of growth and viability of cells in culture. Methods in Enzymology 58: 141.

Rotman, B. and Papermaster, B.W. 1966. Membrane properties of living mammalian cells as studied by enzymatic hydrolysis of fluorgenic esters. Proc. N.A.S.-Biochem. 55: 134.

Seglen, P.O. 1979. Hepatocyte suspensions and cultures as tools in experimental carcinogenesis. J. Toxicol. Environ. Health 5: 551.

Smith, G.C., Purser, D.E., Savell, J.W., Stiffler, D.M., and Dutson, T.R. 1983a. Effect of freezing temperature on weight loss and quality characteristics of beef liver, kidney, heart, and tongue. J. Food Sci. 48: 1726.

Smith, G.C., Savell, J.W., Stiffler, D.M., Johnson, D.D., and Vanderzant, C. 1983b. Weight loss and quality characteristics of frozen versus chilled beef livers and hearts. J. Food Sci. 48: 1728.

Steel, R.G.D. and Torrie, J.H. 1980. "Principles and Procedures of Statistics." McGraw-Hill Book Company, Inc., New York.

Turczyn, M.T. 1980. Guidelines for packaging frozen edible offal for export. U.S. Department of Agriculture. Marketing Research Report Number 1115.

Van De Werve, G. 1980. Isolation and characteristics of hepatocytes. Toxicology 18: 179.

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Latyshev, V.P. 1978. Changes in specific heat and enthalpy of beef liver during chilling and freezing down to 77° K. Proc. 24th European Meeting Meat Research Workers, Kulmbach, W. Germany, Paper D16 (In Russian).

Lorentzen, G. 1974. Design of refrigerant recirculation systems with a special view to their use for contact freezers on board fishing vessels. Annexe 1974: 1; Bull. Int. Inst. Refrig. 125.

MacFarlane, P.G. 1963. Thermal conductance of some packaging materials. Report No. 80, Meat Industry Research Institute of New Zealand, Hamilton, N.Z.

Mawson, R.F. and Collinson, B.R. 1977. Evaluation of lightweight boards for boneless beef cartons. Report No. 565, Meat Industry Research Institute of New Zealand, Hamilton, N.Z.

Meat and Livestock Commission. 1984. U.K. Market Review No. 1. Bletchley, U.K.

Paul, A.A. and Southgate, D.A.T. 1978. McCance and Widdowson's "The Composition of Foods," 4th ed. HMSO London.

Plank, R. 1941. Beitrage zur Berechnung und Bewertung der Gefriergeschwindigkeit von Lebensmitteln. Beihefte Z. ges. Kalte-Industrie Reihe 3 Heft 10. 24 p.

Poppendiek, H.F., Greene, N.D., Morehuse, P.M., Randall, R., Murphy, J.R., and Morton, W.A. 1966. Annual Report on thermal and electrical conductivities of biological fluids and tissues. ONR Contract No. 4095(00), A-2 ASTIA (American Scientific and Technical Information Association) 630 712.

Robinson, J.E. Unpublished data. cited by Morley, M.J. 1972. "Thermal Properties of Meat: Tabulated Data." Meat Research Institute Special Report No. 1., Langford, Bristol, U.K.

Siebel, J.E. 1892. Specific heat of various products. Ice and Refrigeration, April 1892, cited in ASHRAE Handbook — Fundamentals (1977). Ch. 29. Pub. ASHRAE, New York.

Sweat, V.E. 1975. Modelling the thermal conductivity of meats. Trans. ASAE (American Society of Agricultural Engineers) 18: 564.

Templeton, J. and Nicholson, F.J. 1972. Heat transfer and flow characteristics of freezer plates refrigerated with trichloroethylene, calcium chloride brine and pump-circulated Refrigerant 22. Bull. Int. Inst. Refrig. 52: 1083.

Effect of Needle Tenderization on Salt Absorption, Yields, Composition and Palatability of Dry-Cured Hams Produced from Packer Style and Skinned Green Hams

J. D. KEMP and J. D. FOX

─────ABSTRACT─────

The effects of needle tenderization on the curing processes and final cured ham properties of packer style and skinless hams were investigated. Skinless hams absorbed salt faster and lost weight faster than packer style hams. Needle tenderized hams also absorbed salt faster and had more weight loss than nontenderized hams in both the packer style and the skinless groups. Two months' aging resulted in excess dehydration and lowered quality in the skinless tenderized group. In skinless hams, curing, salt equalization and aging times were reduced so that acceptable, though mild flavored, hams that met the USDA requirements for weight loss and salt content were produced within 5 wk.

INTRODUCTION

THE PRODUCTION of dry-cured hams in Kentucky and the Southeastern states has grown substantially over the last several years. Standards for country hams are defined by the USDA (Mussman, 1977) and include the stipulations that hams containing nitrate and/or nitrite must lose at least 18% of uncured weight, must be held at a combination of time and temperature to render them free of live trichinae and the finished product must have an internal salt content of at least 4%. These regulations are based on the assumption that the hams are to be cured intact and with no means of accelerating the penetration of curing ingredients. If methods can be devised to accelerate cure penetration and weight loss during curing, the times for curing and aging could be reduced and a savings in processing costs should be achieved. If the hams thus produced are acceptable to consumers and the shortened procedure is acceptable to the USDA the consumer would also benefit if some of the producers' savings were passed on.

Montgomery et al. (1976) showed that skinless hams had a greater uptake of salt during curing. Mechanical tenderization by the injection of blunt needles into muscles (needle tenderization) is used commercially for fresh meats. The needles disrupt the muscle fibers and connective tissue and leave tiny holes in the muscles. Preliminary work at this station (Leak et al., 1984) using boneless hams has shown that needle tenderization accelerates cure uptake, allows faster weight loss and results in improved tenderness. Marriott et al. (1984), however, reported no significant advantages in using needle tenderization. This study was designed to determine the effect of needle tenderization prior to curing of intact packer style and skinless hams on cure uptake, weight loss, tenderness, composition and palatability traits of dry-cured aged hams.

MATERIALS & METHODS

Experiment 1

Forty-eight frozen hams ranging in weight from 7.4 – 9.9 kg (avg 8.4 kg) were placed in a 3°C cooler and allowed to thaw for 3 days. They were then divided into 4 groups of 12 hams each which re-

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ceived the following treatments.

Group 1 – Controls. Packer style hams (skin and small amount of fat removed from butt portion) were cured by the standard Univ. of Kentucky procedure.

Group 2 - Packer style hams were passed twice through a needle tenderizer (Ross Industries, Midland, VA) – once with the cushion side up and once with the skin side up and cured. The needles had no problem penetrating the skin.

Group 3. the hams were skinned, trimmed to approximately 1 - 2 cm of fat and cured.

Group 4. The hams were skinned as in group 3 and needle tenderized as in group 2.

All hams were cured using 8 kg of curing mixture per 100 kg of ham applied in three applications at day 0, day 3 and day 7. The curing mixture contained 83.4% salt, 13.9% white sugar, 1.7% potassium nitrate and 0.9% sodium nitrite. The hams remained in cure at 2 – 3°C 4 wk and were then placed in a salt equalization room at 10 – 13°C for 18 days. Hams were rinsed, placed in a smokehouse at approximately 38°C and smoked intermittently, using hardwood sawdust as the smoke source, for 2 days after which they were placed in an aging room at 24°C and held until sliced for evaluation.

While in cure each group of hams was divided into sub-groups. Sub-groups 1A, 2A, 3A and 4A contained 7 hams each and were cured as previously described and aged 8 wk. Weights were recorded after curing, after salt equalization, after smoking and after 4 and 8 wk aging.

Sub-groups 1B, 2B, 3B and 4B contained 5 hams each which were cored in the cushion and heel areas at days 14, 21, 28 and 35. Cores were taken with a 2.54 cm coring device by boring approximately 5 cm into the ham. The cores were divided into top, center and inner portions and each portion of each core was analyzed for salt and nitrite (AOAC, 1980). The core holes were filled with melted acetylated monoglyceride (Eastman Chemical Co., Kingsport, TN) which solidified and aided in the prevention of dehydration, and partially curtailed the development of internal mold. After six weeks all hams in these sub-groups were sliced and the inside cushion, center cushion and outside cushion portions were also analyzed for salt and nitrite. After hams in sub-groups 1A, 2A, 3A and 4A had been aged at 24°C for 8 wk, they were sliced and evaluated subjectively for color, aroma and general appearance, and the lean portion of a center slice was analyzed for salt, nitrite and water. Slices of 1.27 cm thickness were broiled and served to a trained dry-cured ham palatability panel which evaluated them for tenderness, flavor intensity, flavor preference, saltiness and overall satisfaction. Ten panelists were used with at least 8 being present at each evaluation session. Eight-point scales were used: tenderness, 1 = extremely tough, 8 = mushy; flavor intensity, 1 = extremely bland, 8 = extremely intense; saltiness, 1 = devoid, 8 = extremely salty; flavor preference and overall satisfaction, 1 = dislike extremely, 8 = like extremely. A 2.54 cm slice from each ham was broiled and a shear test was performed using a Warner-Bratzler shear. Data were analyzed using the Statistical Analysis System (SAS, 1984).

Experiment 2

The procedure for Exp. 2 was influenced by the results of Exp. 1, which showed that in both nontenderized and tenderized hams the rate of salt uptake was more rapid in skinless hams; therefore only skinless hams were used. Twenty-four frozen hams were thawed and skinned as in Exp. 1. The curing formula and the curing, salt equalization and aging temperature were the same. However, only 7 kg of cure was used per 100 kg hams and the cure was applied in two applications on day 0 and day 4 rather than in three applications as in Exp. 1. Hams were placed in four groups of six hams each as follows:

Group 1. Not tenderized. Cured 1 wk, left in salt equalization 1 wk, smoked 1 day and aged a minimum of 2 wk or until yield was 82% or less.

Group 2. Tenderized and treated like Group 1.

Group 3. Not tenderized. Cured 2 wk, left in salt equalization 1 wk, smoked 1 day and aged as in group 1.

Group 4. Tenderized and treated like group 3.

Hams in groups 1 and 2 were weighed after curing, after salt equalization, after smoking, after 2 wk aging (total time, 4 wk) and weekly thereafter until the yields were 82% or less. Hams in groups 3 and 4 were weighed similarly except they were weighed after 1 wk aging (total time, 4 wk) and weekly thereafter until the yield was 82% or less. After reaching the desired yield all hams were sliced: evaluated for color, aroma, firmness and general appearance; analyzed for salt, nitrite and water (AOAC, 1980); and examined for sensory properties and for tenderness as in Exp. 1. Eight of a total of 9 panelists were present for each session. Statistical analysis also was similar to that used in Exp. 1.

RESULTS & DISCUSSION

Experiment 1

Mean salt percentages for the outside, middle and inner portions of the cores from the ham cushions at 2 through 6 weeks for each treatment group are given in Table 1 and for the inner portion in Fig. 1. There were no significant differences between treatment means in the outside portion of the core at either time period. This was as expected as surface salt reflected the equal amount applied to each group. There was generally a decrease in salt for 2 – 5 wk and an increase at 6 wk probably due to moisture loss in the surface area. The important areas, however, were the middle and inner areas particularly the inner part of the core. Some minor differences were noted for the middle core area especially the increase in the 6 wk skinless (4.50%) and skinless tenderized group (6.17%). The most important

differences were in the inner part of the core (Fig. 1). Note the time it took for each group to reach 4% salt. This was 6 wk for the control group, 5 wk for the control tenderized group, 6 wk for the skinned group but only 4 wk for the skinned tenderized group. Thus, skinning plus tenderization shortened salt absorption time by 2 wk.

Most country hams are cured with the skin on the heel or shank portion. The skin slows salt absorption and the 4% level is achieved more slowly in the shank than in the cushion part. Table 2 shows the salt data for all portions of the cores from the shank. Note that the 4% salt level is not achieved at any depth at any time period for the control group or the control tenderized group indicating that 6 wk is insufficient time for complete salt equalization. In the skinless hams, however, the 4% level was reached in the middle core portion in 4 wk in the skinless group and 3 wk
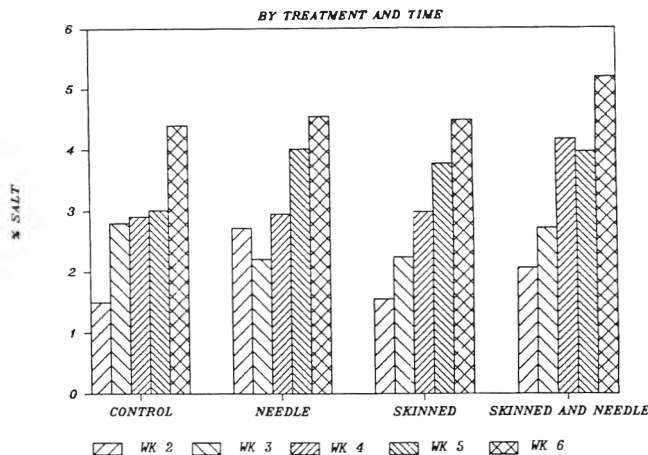
Fig. 1–Percent salt in center of cushion – by treatment and time.

Table 1–Salt percentages by depth[a], treatment[a], and time[a] – cushion, Exp. 1

	Depth 1 — Outside				Depth 2 — Middle				Depth 3 — Inner			
	Treatment				Treatment				Treatment			
Time (wk)	1 B Control	2 B Control tenderized	3 B Skinless	4 B Skinless tenderized	1 B Control	2 B Control tenderized	3 B Skinless	4 B Skinless tenderized	1 B Control	2 B Control tenderized	3 B Skinless	4 B Skinless tenderized
2	7.70[b]	6.67[b]	4.93[b]	5.63[b]	4.01[bc]	5.26[b]	3.14[b]	3.79[bc]	1.50[b]	2.72[b]	1.56[b]	2.06[b]
3	6.11[b]	5.72[b]	4.64[b]	5.06[b]	4.65[b]	4.04[b]	3.38[b]	3.99[b]	2.80[b]	2.21[b]	2.24[b]	2.72[b]
4	5.55[b]	5.21[b]	4.66[b]	6.31[b]	4.12[b]	4.04[b]	3.94[b]	4.88[b]	2.91[b]	2.95[b]	2.98[b]	4.17[c]
5	4.44[b]	5.96[b]	4.36[b]	5.25[b]	4.02[b]	5.20[b]	4.09[b]	4.71[b]	3.01[b]	4.01[b]	3.77[b]	3.96[b]
6	5.47[b]	6.18[b]	4.62[b]	6.27[b]	5.15[bc]	5.49[bc]	4.50[b]	6.17[c]	4.40[b]	4.54[b]	4.48[b]	5.18[b]

[a] ANOVA — Sig (P < 0.01) for depth, treatment, and time. Interactions sig (P < 0.01) for trt x depth, trt x time and depth x time.
[b,c] Treatment means in rows within depth and time with different superscripts are different (P < 0.05).

Table 2–Salt percentages by depth[a], treatment[a], and time[a] – shank, Exp. 1

	Depth 1 — Outside				Depth 2 — Middle				Depth 3 — Inner			
	Treatment				Treatment				Treatment			
Time (wk)	1 B Control	2 B Control tenderized	3 B Skinless	4 B Skinless tenderized	1 B Control	2 B Control tenderized	3 B Skinless	4 B Skinless tenderized	1 B Control	2 B Control tenderized	3 B Skinless	4 B Skinless tenderized
2	2.26[b]	2.02[b]	4.25[c]	5.32[c]	1.23[b]	1.60[bc]	2.28[c]	3.30[d]	0.89[b]	1.72[c]	1.33[bc]	2.43[d]
3	2.44[b]	2.38[b]	4.27[c]	6.05[b]	1.69[b]	1.80[bc]	2.97[c]	4.97[d]	1.28[b]	1.65[b]	2.21[b]	4.37[c]
4	2.73[b]	2.76[b]	5.55[c]	6.86[c]	2.42[b]	2.28[b]	4.63[c]	5.62[c]	1.92[b]	2.21[bc]	3.90[c]	4.51[c]
5	3.05[b]	3.53[b]	4.85[c]	6.15[d]	2.57[b]	3.09[b]	4.08[c]	5.51[d]	2.24[b]	2.78[bc]	3.64[c]	4.82[c]
6	3.02[b]	3.39[b]	4.57[c]	6.36[d]	2.77[b]	3.30[b]	4.38[c]	6.13[d]	2.84[b]	2.83[b]	4.20[c]	5.74[d]

[a] ANOVA — Sig (P < 0.01) for depth, treatment, and time. Interactions sig (P < 0.01) for trt x depth, trt x time and depth x time.
[b,c,d] Treatment means in rows within depth and time with different superscripts are different (P < 0.05).

in the skinless-tenderized group. In the inner critical portion (Fig. 2) the 4% level was approached (3.90%) in 4 wk in the skinless group and reached in 3 wk in the skinless-tenderized group. Thus, the greatest overall effect on salt absorption was in the skinless tenderized group, showing the beneficial effect of skinning and tenderizing on salt absorption.

Nitrite levels (Tables 3 and 4) were influenced ($P < 0.05$) by depth, treatment and time. The outer samples at all time periods had higher levels than the middle and inner portions of the cores. Tenderized hams had higher levels than nontenderized hams in both skin-on and skinless hams showing that tenderizing enhanced nitrite absorption. There was no consistent pattern due to time. Since nitrate as well as nitrite was used the uneven breakdown of nitrate could have caused the uneven levels of nitrite. In all cases sufficient nitrite was present for it to serve its functions, but in no case was the mean level for any group at 5 or 6 weeks above the USDA approved level of 200 ppm. The data, therefore, in Exp. 1 showed that both skinning and needle tenderization caused an accelerated rate of salt and nitrite absorption.

Yield, subjective scores, composition, palatability, panel scores and shear values for the hams in groups 1A, 2A, 3A and 4A are given in Table 5. Ham yields are influenced by time in curing and aging, lean content of the ham, lean surface area exposed, temperature and humidity of aging, and anything else that would affect moisture movement to the surface. The latter could be caused by needle tenderization. The hams used here were similar in lean content before skinning and were cured and aged for the same time and at the same temperature and humidity. The variables, therefore, that should affect yield time were skinning and tenderizing. The effect of skinning on yield was noticeable ($P < 0.05$) after 4 wk in cure and the difference widened as

curing and aging progressed. Removal of the skin allowed moisture to move more freely to the surface and thus evaporate. Needle tenderization of the unskinned hams caused a decrease ($P < 0.05$) in yield after 2 wk in salt equalization and throughout curing and aging. The small holes made by the needles apparently allowed moisture to migrate to the surface. In the skinless hams the mean for the tenderized hams at each period was lower than for the nontenderized group: in fact, at 4 and 8 wk there was a difference of 2.8 percentage points. However, because of variation, the differences were not significant. Overall, both skinning and tenderizing decreased yields. From an economic standpoint the yields of the skinless and skinless tenderized were excessive and had a marked effect on some of the other ham characteristics.
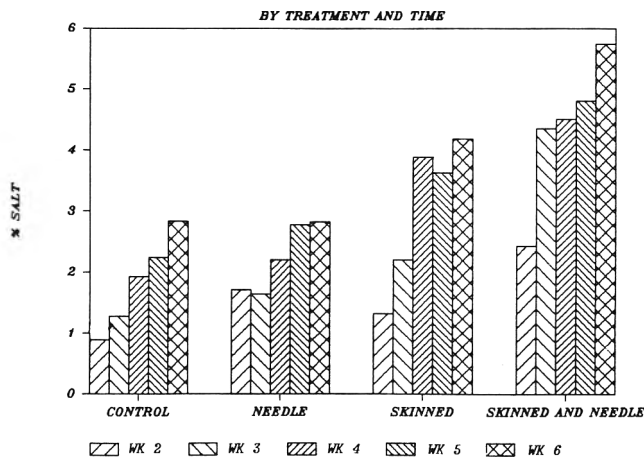
Fig. 2—Percent salt in center of shank — by treatment and time.

Table 3—Nitrite (ppm) by depth[a], treatment[a], and time[a] — cushion, Exp. 1

| | Depth 1 — Outside | | | | Depth 2 — Middle | | | | Depth 3 — Inner | | | |
| | Treatment | | | | Treatment | | | | Treatment | | | |
Time (wk)	1 B Control	2 B Control tenderized	3 B Skinless	4 B Skinless tenderized	1 B Control	2 B Control tenderized	3 B Skinless	4 B Skinless tenderized	1 B Control	2 B Control tenderized	3 B Skinless	4 B Skinless tenderized
2	256.97[b]	267.29[b]	191.59[b]	231.35[b]	64.40[b]	80.66[b]	38.77[b]	58.24[b]	13.04[b]	29.34[b]	11.63[b]	18.70[b]
3	189.55[bc]	196.65[bc]	145.31[b]	302.07[b]	52.14[bc]	50.33[bc]	32.05[b]	77.88[c]	15.95[b]	16.58[b]	18.78[b]	45.04[c]
4	226.17[b]	396.93[b]	227.98[b]	293.68[b]	76.95[b]	201.63[c]	129.69[bc]	168.18[bc]	48.21[b]	136.17[c]	86.33[bc]	146.49[c]
5	105.77[b]	162.63[b]	85.07[b]	72.48[b]	27.13[a]	78.92[a]	31.79[a]	34.99[a]	10.28[b]	18.02[b]	30.44[b]	22.16[b]
6	141.37[a]	199.70[a]	122.00[a]	181.74[a]	57.39[b]	182.99[c]	63.61[b]	99.97[b]	30.56[b]	149.49[c]	54.97[b]	71.29[b]

[a] ANOVA — Sig ($P < 0.01$) for depth, treatment, and time. Interactions sig ($P < 0.01$) for trt x depth, trt x time and depth x time.
[b,c] Treatment means in rows within depth and time with different superscripts are different ($P < 0.05$).

Table 4—Nitrite (ppm) by depth[a], treatment[a], and time[a] — shank, Exp. 1

| | Depth 1 — Outside | | | | Depth 2 — Middle | | | | Depth 3 — Inner | | | |
| | Treatment | | | | Treatment | | | | Treatment | | | |
Time (wk)	1 B Control	2 B Control tenderized	3 B Skinless	4 B Skinless tenderized	1 B Control	2 B Control tenderized	3 B Skinless	4 B Skinless tenderized	1 B Control	2 B Control tenderized	3 B Skinless	4 B Skinless tenderized
2	42.69[b]	39.18[b]	96.06[bc]	194.82[c]	18.77[b]	22.79[b]	27.17[b]	41.04[b]	6.18[b]	15.68[bc]	11.16[bc]	21.93[c]
3	34.35[b]	32.71[b]	68.92[b]	199.01[c]	13.88[b]	17.76[b]	27.11[bc]	128.94[c]	8.39[b]	14.34[b]	25.61[b]	59.74[c]
4	80.99[b]	151.31[b]	220.13[bc]	337.84[c]	89.94[b]	112.49[b]	188.62[b]	131.57[b]	51.20[b]	104.99[bc]	150.46[c]	143.84[c]
5	31.46[b]	31.47[b]	82.07[b]	83.93[b]	13.51[b]	14.55[b]	37.18[bc]	61.06[c]	7.57[b]	12.40[b]	25.27[bc]	36.30[c]
6	34.18[b]	145.14[c]	77.29[bc]	147.16[c]	30.10[b]	121.98[c]	52.73[c]	112.73[c]	29.07[b]	94.16[c]	55.13[b]	92.63[c]

[a] ANOVA — Sig ($P < 0.01$) for depth, treatment and time. Interaction Sig ($P < 0.01$) for trt. x depth, trt. x time, trt. x depth x time.
[b,c] Treatment means in rows within depth and time with different superscripts are different ($P < 0.05$).

Table 5—Mean yields, subjective scores, mean composition, panel scores and shear values — Exp. 1

Group	Treatment	No.	2 wk in cure	4 wk in cure	2 wk in salt equalization	4 wk in salt equalization	Out of smoke	Aged 4 wk	Aged 8 wk	Color	Aroma	General appearance	Salt %	NO$_2$ ppm	H$_2$O %	Tenderness	Flavor intensity	Flavor preference	Salt	Overall satisfaction	SM[f]	ST[f]	BF[f]
			Yields, % in cure wt							Subjective scores[d]			Composition			Panel scores[e]					Shear values Kg/2.54 cm core		
1A	Control	7	95.7a	94.2a	87.0a	85.6a	84.8a	79.5a	76.1a	3.3	3.4a	4.0a	5.45a	17.87a	55.07a	5.31a	5.82a	5.88a	5.42a	5.90a	7.45a	6.92a	11.68a
2A	Control Tenderized	7	93.6ab	92.1ab	83.7b	82.1b	81.1b	74.7b	71.3b	3.3	3.2a	3.4b	7.33bc	26.47a	51.86b	4.69ab	5.85a	5.21c	5.83b	5.19bc	8.16ab	9.97ab	11.25a
3A	Skinless	7	93.0ab	91.4b	82.5bc	80.6bc	79.3bc	71.6b	65.9c	3.5	3.5	3.4b	6.43ab	16.16a	53.50ab	4.54b	5.97a	5.64ab	5.77b	5.44b	10.44b	8.43ab	10.95a
4A	Skinless Tenderized	7	91.9b	90.4b	80.1c	78.1c	76.8c	68.8c	63.1c	3.7a	3.3	3.2b	7.98a	21.51a	50.76b	4.50b	6.06a	5.29bc	6.02b	4.89c	9.87ab	10.75b	9.81a

a,b,c Means within columns with different superscripts are different (P < 0.05).
d Color: 4 = dark red, 3 = red, 2 = light red;
Aroma: 4 = typically aged, 3 = moderately aged, 2 = slightly aged;
Gen. app: 4 = excellent, 3 = good, 2 = fair

e Based on 8 point scale: Tend: 1 = extremely tough, 8 = mushy; Flavor intensity: 1 = extremely bland, 8 = extremely intense; Flavor preference; and Overall satisfaction: 1 = dislike extremely, 8 = like extremely; Saltiness: 1 = devoid, 8 = extremely salty.
f SM = Semimembranosus; ST = Semitendinosus; BF = Biceps Femoris

Table 6—Number[a] of hams achieving 82% or lower yield by weeks of aging — Exp. 2

Group	1	2	3	4
Wk aged	Nontenderized cured 1 wk S.E. 1 wk	Tenderized cured 1 wk S.E. 1 wk	Nontenderized cured 2 wk S.E. 1 wk	Tenderized cured 2 wk S.E. 1 wk
1	0	0	0	4
2	4	5	3	1
3	1	0	1	1
4	0	0	2	0
5	1	1	0	0

a Six hams per group.

Color and aroma scores for the cut hams (Table 5) were similar for all groups and were highly acceptable. General appearance scores, however, were higher (P < 0.05) for the control group with the skinless-needled group having the lowest score. Some of the slices in the skinless or tenderized groups were somewhat hard and dry and thus were not as attractive. Some of the hams in the tenderized group also had noticeable "pin holes" where the needles had penetrated which detracted slightly from general appearance.

Salt and moisture content was affected (P < 0.05) by treatment. In both unskinned and skinned groups the salt content was higher and the moisture content was lower in the tenderized groups reflecting the relationship of weight loss to composition. Nitrite was not affected (P < 0.05) although the means were greater for the tenderized groups.

Panel scores generally favored the control or unskinned non tenderized group. Since this group was higher in moisture, softer and less salty, the panelists considered it more tender with higher flavor preference and overall satisfaction scores. Lowest scores were noted for the skinless tenderized group which was the driest and saltiest.

Shear values were lower (P < 0.05) for the semimembranosus (SM) and semitendinosus (ST) muscles, again reflecting the effect of the softer higher moisture tissue. There were no significant differences in shear values of the biceps femoris (BF) muscle which isn't surprising since it is well protected by fat and did not dehydrate to the extent of the other muscles.

Exp. 1 thus showed that faster salt penetration and more rapid weight loss was achieved by skinning and needle tenderization, but if the hams were aged for one or two months the lowered yields caused by excess weight loss made them less desirable than the normally cured hams. Thus, Exp. 2 was designed, based on results of the above data, to cure and age for the minimum time in order to achieve the legal limits of salt and weight loss.

Experiment 2

Only skinless hams were used. Half were not tenderized. Since the data from Exp. 1 showed that the salt level was approached or attained in approximately 3 weeks in the shank of the tenderized hams, the experiment was designed to have a combination of curing at 3°C for 1 or 2 wk, salt equalization at 13°C for 1 wk and an aging time at 24°C for 2 wk or until the ham yields were 82% or less.

Table 6 shows the time necessary to reach the 82% or lower yield. With one exception the tenderized hams had reached the yield goal of 82% at the end of 3 wk of aging or within 4 total wk for group 2 and 6 total wk for group 4. Salt levels for these hams after attaining the 82% yield (Table 7) were 4.2% for group 2 and 4.5% for group 4 indicating that this time might be sufficient to produce an acceptable product that meets the USDA standards for salt, weight loss and trichinae control except that the time in cure is less than recommended (Houston, 1983).

As noted in Table 7, yields of hams that were held in cure 2 wk and in salt equalization 1 wk had lower yields after salt equalization (P < 0.05) and after 1 wk of aging than those held in cure only 1 wk, showing the effects of time. There were no significant differences in subjective scores with color generally being red; aroma, slightly aged; firmness, slightly firm; and general appearance, slightly above good. The aroma score was a matter of concern as the hams had not developed the aroma desired in a country ham. A few hams also were in the slightly soft category which indicated that they needed further aging.

Composition data showed that the mean levels for moisture exceeded 60% for all groups with no significant difference between groups. This level of moisture exceeds that normally present in typical aged hams (Kemp et al., 1978) but are similar in moisture content when hams are vacuum packaged after attaining a yield of 80 to 81% (Kemp et al., 1981). Nitrite levels were acceptable but no affected by treatment.

Panel scores were similar for all traits except flavor intensity. The nontenderized hams were less intense in flavor (P < 0.05) for the shorter cured group than for either tenderized group. There were no differences in shear values due to treatment within either muscle. The shear values were considerably lower, however, than most equivalent shears in Exp. 1 (Table 5).

The overall data thus show that skinning and needle tenderization will allow faster salt absorption and faster weight loss than conventional hams or skin-on tenderized hams. The fast cured short aged hams, however, were less intense in flavor and slightly softer than normal hams. It is believed that further controlled aging where additional weight loss is kept to a minimum will allow a much shorter curing and salt equalization time to become practical.

Table 7—Ham characteristics as affected by curing and aging time — Exp. 2

Group Treatment	1 Cured 1 wk Salt equalization 1 wk	2 Cured 1 wk Salt equalization 1 wk — Tenderized	3 Cured 2 wk Salt equalization 1 wk	4 Cured 2 wk Salt equalization 1 wk — Tenderized
No. of hams	6	6	6	6
Yields, %				
Cured	94.7	97.1	97.1	96.4
Salt equalization	94.8[a]	94.2[a]	88.8[b]	88.3[b]
Smoked	88.7	87.6	87.3	86.8
Aged 1 wk	84.2	83.8	82.9	81.3
Subjective scores[c]				
Color	3.4	2.9	3.1	2.9
Aroma	1.7	1.8	2.2	1.6
Firmness	3.0	3.0	2.9	3.1
General appearance	3.6	3.2	3.3	3.2
Composition				
H_2O, %	62.6	61.2	63.0	60.7
Salt, %	3.8	4.2	3.7	3.5
Nitrite, ppm	87.4	70.2	58.6	89.1
Panel scores[d]				
Tenderness	5.3	5.5	5.1	5.4
Flavor intensity	5.5[a]	6.0[b]	5.8[ab]	6.0[b]
Flavor preference	5.5	5.5	5.4	5.4
Saltiness	5.2	5.5	5.2	5.6
Overall satisfaction	5.5	5.5	5.4	5.4
Shear, kg/2.54 cm				
SM	7.6	6.3	6.8	7.1
ST	6.8	4.5	5.8	5.5
BF	7.2	6.3	7.2	7.7

[a,b] Means on the same row with different superscripts are different (P < 0.05).

[c] Color: 4 = dark red, 3 = red, 2 = light red; Aroma: 4 = typically aged, 3 = miderately aged, 2 = slightly aged, 1 = no aged aroma. Firmness: 4 = firm, 3 = slightly firm, 2 = slightly soft, 1 = soft; Gen. Appearance: 4 = excellent, 3 = good, 2 = fair, 1 = poor.

[d] Based on 8-point scale: Tenderness: 1 = extremely tough; 8 = mushy; Flavor intensity: 1 = extremely bland, 8 = extremely intense; Flavor Preference and Overall Satisfaction: 1 = dislike extremely, 8 = like extremely; Saltiness: 1 = devoid, 8 = extremely salty.

REFERENCES

AOAC. 1980. "Official Methods of Analyses," 13th ed. Association of Official Analytical Chemists, Washington, DC.

Houston, D.L. 1983. Trichinae control requirements. Federal Register 48: 10065.

Kemp, J.D., Langlois, B., and Fox, J.D. 1978. Composition, quality and microbiology of dry-cured hams produced from previously frozen green hams. J. Food Sci. 43: 860.

Kemp, J.D., Langlois, B.E., and Fox, J.D. 1981. Effect of vacuum packaging and potassium sorbate on yield, yeast and mold growth and quality of dry-cured hams. J. Food Sci. 46: 1015.

Leak, F.W., Kemp, J.D., Fox, J.D., and Langlois, B.E. 1984. Effect of boning time and mechanical tenderization on the quality and microflora of tumbled dry-cured hams. (Abstract). J. Anim. Sci. Supp. 1: 231.

Marriott, N.G., Kelly, R.F., Shaffer, C.K., Graham, P.P., and Boling, J.W. 1984. The effect of blade tenderization on the cure and sensory characteristics of dry-cured hams. Proc. European Meeting of Meat Research Workers: 264.

Montgomery, R.E., Kemp, J.D., and Fox, J.D. 1976. Shrinkage, palatability and chemical characteristics of dry-cured ham as affected by skinning procedure. J. Food Sci. 41: 1110.

Mussman, H.C. 1977. Definitions and standards of identity or composition — "Country," "Country Style," or "Dry-Cured" hams and pork shoulders. Federal Register 42: 3298.

SAS. 1984. Users Guide. Statistical Analysis System Institute, Cary, NC.

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Correlations of Sensory and Instrumental Evaluations of Roast Beef Texture

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─────────────── ABSTRACT───────────────

Beef semitendinosus roasts heated to endpoint temperatures of 60°, 70°, and 80°C were evaluated by a taste panel and by instrumental tests of shear, penetration, and compressive force. Correlations were found between sensory parameters of chewiness, hardness and tenderness and between these parameters and shear parameters of firmness and cohesiveness. Compression-hardness was related positively to panel scores for moisture and negatively to mealiness scores. Compression-cohesiveness was related only to panel scores for oiliness while compression-springiness and penetration hardness were not related to any of the sensory parameters evaluated.

INTRODUCTION

FOOD TEXTURE has been defined as "the composite of those properties which arise from the structural elements, and the manner in which it registers with the physiological senses" (Szczesniak, 1963). This definition recognizes three essential elements of texture: (1) that it is the result of the structure of the food; (2) that it is a composite of several properties; and (3) that it is a sensory quality. In the measurement of food texture, therefore, these elements must be considered. From the above definition, it could be deduced that humans are the best instrument for evaluating food texture, since only humans can perceive, analyze, integrate, and interpret a large number of textural sensations at the same time (Larmond, 1976). All sensory evaluation, regardless of whether it involves laboratory panels or consumers, is faced with many methodological, psychological, and physiological problems. In addition, it is both costly and time consuming. To avoid these problems and to obtain the advantages of speed, reproducibility, and relative ease of standardization, instrumental methods of measuring the mechanical aspects of food texture were sought. Because of the complex nature of food texture, it was recognized that it is important to measure as many of a food's textural parameters as possible. This may be accomplished either by using several different tests, each detecting one or more parameters, or by using one test from which a number of parameters may be characterized. Because of its greater efficiency, the latter method has gained general acceptance.

For meat, texture has come to be synonymous with tenderness and consumer research has determined that tenderness is the single most important factor in assessing meat acceptability. The measurement of meat tenderness is extremely difficult because meat is not a simple one-component system (Cover et al., 1962). It is instead the result of two structural components, muscle fibers and connective tissue, and is further complicated by the presence of fat interspersed within these structural elements. Since the pioneering work by Cover et al., almost all sensory evaluations of meat tenderness have involved some form of multicomponent analysis. This has not been the case with instrumental techniques.

Traditionally, the most common instrument for evaluating meat tenderness has been the Warner-Bratzler shear. Reports of correlations between shear values, measured as maximum shear force, and taste panels have ranged from highly significant to no significance (Szczesniak, 1968). Larmond and Petrasovits (1972) proposed using two measurements from the Warner-Bratzler, peak force and slope of the shear force-time curve. They found that the extent of the relationship between these measurements and panel evaluations was dependent on the nature of the sample. Bouton et al. (1971) developed a compression test for assessing meat tenderness. They reported that this test was highly correlated with taste panel measurements; however, the factors evaluated by the panel were not defined. In addition, correlations between this test and Warner-Bratzler shear were found to vary, depending on the temperature of the meat being tested. Rhodes et al. (1972) investigated the relationship between taste-panel evaluations of tenderness and juiciness and characteristics related to deformation, force, and work measured during compression of meat samples. They concluded that correlations between the panel and the various instrumental parameters were not significantly improved over those from a one-component Warner-Bratzler shear test. Bouton et al. (1975) analyzed the force-deformation curves produced during shear, compression, and tensile tests of meat samples. They hypothesized that the initial effect of applied force was probably to produce a yield in myofibrillar structure and then later applied force probably was resisted by connective tissue. Tenderness characteristics of beef semitendinosus roasts heated in ovens were compared with those of small samples heated as cylindrical cores in glass tubes in a water bath through multicomponent analysis of the force-deformation curves produced during shear and penetration testing (Brady and Penfield, 1982). Correlation coefficients indicated that the magnitude and direction of the relationship between sensory and instrumental tests varied according to whether samples were heated as intact roasts or as cores.

Because of the importance of texture to meat acceptance, it is important to understand the components contributing to this characteristic and methods for measuring these. In the present study a trained sensory panel was used to identify and quantify factors related to meat tenderness and the effect of endpoint temperature on these. Tenderness of the meat also was evaluated using Warner-Bratzler shear, penetration, and compression techniques. Correlation coefficients were calculated to determine the relationships among sensory parameters, among instrumental parameters, and between sensory and instrumental parameters.

MATERIALS & METHODS

TWELVE CHOICE-GRADE beef semitendinosus muscles were obtained from the Meat Science Laboratory, University of Illinois, Urbana. Four muscles were randomly assigned to each of three endpoint temperatures, 60°, 70°, or 80°C. Roasts were wrapped in freezer paper, frozen at −30°C, then stored at −18°C for periods of no longer than 6 wk.

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Sample preparation and heating

Samples were randomly assigned to test days such that samples representing two different endpoint temperatures were heated on each test day. After thawing for 48 hr at 4°C, roasts were placed in a preheated 165°C electric oven and heated until a copper-constantan thermocouple placed in the center of the roast registered the desired endpoint temperature. When the endpoint temperature was reached, roasts were removed from the oven, covered loosely with foil, and cooled at 26°C for 30 min. Appropriate weights were determined before and after heating for use in calculation of losses. Roasts were cut across the muscle fibers into 6.15-cm sections and cores 2.5 cm in diameter, cut parallel to the muscle fibers, were removed. Cores were randomly assigned for testing by either the sensory panel or by one of the instrumental procedures. Tissue remaining after the removal of the cores was ground and used for moisture determinations.

Moisture determination

A modified AOAC (1980) method was used for moisture analysis. Triplicate 3 – 5g finely ground samples of meat were dried overnight in a 45°C oven. Following an additional 2 hr of drying in a 100°C oven, samples were weighed and the percent moisture content calculated.

Sensory testing

Nine sensory panelists were recruited from the students and staff of the Dept. of Foods & Nutrition. Panelists were trained in a series of nine sessions, each 1 hr long, occurring over a period of 5 wk. In the first five of these, panelists were taught the textural characteristics defined by Szczesniak (1963) and procedures for evaluating these. Samples representing levels of these parameters were provided to allow panelists to practice using the terms (Civille and Szczesniak, 1973). During session six, panelists applied the principles learned in the previous sessions to develop a ballot specifically for use in evaluating meat. Remaining training sessions were devoted to testing and refining this ballot.

The final ballot consisted of nine characteristics. Each characteristic was evaluated on a 15 cm unstructured line scale anchored 1 cm from each end with terms representing extremes of the characteristic. Characteristics evaluated, guidelines for evaluating each characteristic, and anchor terms for each are presented in Table 1.

Sensory test sessions were conducted immediately after cores were removed from heated roasts. During test sessions, panelists worked in individual booths. They were presented with samples from each of that day's endpoint temperature treatments separately and order of presentation of the treatment samples was randomized among panelists. Samples for each treatment consisted of two core sections each 2.5 cm in length served on white china plates. Two cores per treatment were served to assure panelists had adequate amounts of samples to complete their evaluations. Panelists were given unsalted crackers and room-temperature water to cleanse the palate before presentation of samples from each treatment. All tests were carried out under green light to disguise any color differences due to the different endpoint temperatures. Following

panelists' evaluations, values were assigned to ratings of each parameter by measuring the distance, in centimeters, of the panelists' mark from the left end of the line scale.

Instrumental tests

Instrumental tests for texture characteristics were conducted on each treatment on day of heating using an Instron Universal Test Machine, Model 1132. A 50-kg load cell was used with crosshead and chart speeds of 20 cm/min. Samples were sheared by cutting across the fibers with a Warner-Bratzler shear attachment. Resultant curves were evaluated for parameters of "firmness" and "cohesiveness" (Larmond and Petrasovits, 1972). "Shear-Firmness" was calculated as the slope of a line drawn from the origin of the curve to its peak and was reported in kilograms per minute. "Shear cohesiveness," expressed in kilograms, was measured as the peak force recorded on the shear-deformation curve. "Penetration-hardness" was measured as the force, in kilograms, required to drive a flat probe 0.5 cm in diameter vertically 80% of the way through a sample. Samples were presented so that the fibers were perpendicular to the direction of the plunger movement (Bouton et al., 1971). Core segments, 2.54 cm in length, were compressed twice to 80% of their height using a flat plunger 5.7 cm in diameter. Cores were placed on the test platform so that the direction of compression was parallel to the muscle fibers. Properties of "hardness," "springiness" and "cohesiveness" were evaluated from the resulting two-bite curves (Bourne, 1978). "Hardness" was defined as the peak height of the first curve, in kilograms. "Springiness" was the width of the second curve, expressed in minutes, while "cohesiveness" was the ratio of the area of the second curve to that of the first. Four tests each of shear, penetration, and compression were conducted on randomly selected cores from each roast.

Statistical analysis

Analysis of variance was used to identify differences in the various parameters due to endpoint temperature. When significant differences were found, Tukey's test was used for mean separation. Correlation coefficients were calculated to determine relationships among sensory parameters, among objective parameters, and between sensory and objective parameters.

RESULTS & DISCUSSION

Effect of endpoint temperature

As endpoint temperature was increased, time to reach the designated temperature also was increased (Table 2). Heating losses increased significantly between 60° and 70°C but were not significantly different between 70° and 80°C. This is in contrast to Cross et al. (1976) who found heating losses increased as endpoint temperatures were increased in 10°C increments between 60° and 90°C and increased significantly between 70° and 80°C. In both the present study and that by Cross et al. a major portion

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Table 1—Characteristics and scoring criteria for sensory evaluation of beef semitendinosus

Characteristic	Scoring criteria and guidelines for evaluation	Scale anchors[a]
Springiness	Compress sample between molar teeth and release pressure	Stays compressed; quickly returns
Hardness	Force required to compress the sample between molar teeth	Soft; hard
Deformation	Amount of deformation before rupture, compressing between molar teeth	Slight; extreme
Chewiness	Length of time to prepare sample for swallowing	Low; high
Strand separation	Amount the sample falls apart into strands during chewing	None; great
Mealiness	Amount of hard/dry particles left in mouth after swallowing	Low; high
	After swallowing rate:	
Moisture	Overall impression of moisture content of sample	Dry; moist
Oiliness	Overall impression of oiliness of sample	Low; high
Tenderness	Overall impression of sample's tenderness	Tough; tender

[a] First term anchors left end of scale; second term anchors right.

of the total loss was attributed to evaporation. Moisture analyses of the heated samples revealed no significant differences due to heat treatment (Table 2).

Chewiness, mealiness, and moisture were the only sensory characteristics affected by endpoint temperature (Table 3). Samples heated to 60°C were significantly more chewy, i.e., required a greater length of time to prepare for swallowing, than those heated to 70° or 80°C, but there were no differences in the chewiness of samples heated to the two higher endpoints. These findings are in contrast to those of Martens et al. (1982) who reported that their sensory panel found total chewing work was lowest for beef semimembranosus samples heated to $60 - 63^\circ$C then increased with increasing endpoint temperatures. Heating to 70°C resulted in samples significantly more mealy than those heated to either 60° or 80°C. Collagen solubilization, occurring at $60 - 63^\circ$C, might account for increased mealiness at 70°C (Martens et al., 1982), while subsequent denaturation of actin, occurring at temperatures in the range of $67 - 73^\circ$C, would be associated with the decreased mealiness at the 80°C endpoint. Although no differences were found in moisture contents of the heated roasts, panelists judged samples heated to 60°C more moist than those heated to 70° or 80°C. This corresponds, however, to the effect of endpoint temperature on heating losses (Table 2).

Both shear firmness and cohesiveness were affected by endpoint temperature (Table 4). Samples heated to both 60° and 80°C were significantly more firm than those heated to 70°C. Samples heated to 70°C were less cohesive than those heated to 80°C and both of these higher endpoint temperatures resulted in meat that was less cohesive than samples heated to 60°C. The effects of endpoint temperature on these two shear parameters suggest that the two parameters combined provide a picture of the changes in a number of the muscle components of the samples during heating. Coagulation of contractile proteins with little change in connective tissue is suggested by the

higher firmness and cohesiveness values at the 60°C samples (Paul et al., 1973; Bouton et al., 1981; Marten et al., 1982). Continued heating resulted in collagen solubilization which led to decreased cohesiveness scores although this effect was overcome by the increased coagulation of contractile proteins at the 80°C endpoint (Paul et al., 1973).

Penetration-hardness increased as endpoint temperature was increased while compression-hardness increased significantly between 60° and 70°C but showed a slight but nonsignificant decrease between 70° and 80°C. Values for compression-springiness followed a similar pattern. Compression-cohesiveness was not significantly affected by endpoint temperature. These findings are in contrast to those of Bouton et al. (1975) who observed that penetration and compression tests appeared to be better indicators of connective tissue changes than shear-type tests. In the present study, changes in the compression parameters are not in the directions that would be expected if the effects observed were associated predominantly with connective tissue changes.

Correlations

Examinations of the correlations among the nine sensory parameters revealed that only five of these relationships were significant (n = 12). Overall tenderness showed strong negative correlations with both hardness (r = −0.950, p < 0.01), the force to compress the sample between the molar teeth, and chewiness (r = −0.919, p < 0.01), the length of time required to prepare the sample for swallowing. There was a strong positive relationship between hardness and chewiness (r = 0.887, p < 0.01). The ease with which fiber strands separated during chewing was found to be negatively related to sample oiliness (r = −0.671, p < 0.05), while mealiness, the presence of hard, dry particles in the mouth after swallowing, showed a strong negative correlation with moisture scores (r = −0.932; p < 0.01).

When the various instrumental parameters were compared, the only significant relationship found was between the shear parameters of firmness and cohesiveness (r = 0.768, p < 0.01) and between shear cohesiveness and compression springiness (r = −0.603, p < 0.05).

Correlations between sensory and instrumental tests revealed that the sensory parameters related to work required to masticate the samples, i.e., hardness, deformation, chewiness, and overall tenderness, were all correlated with shear firmness (Table 5) and, with the exception of deformation, these same sensory parameters were related to shear cohesiveness. These sensory parameters were not found to be associated with penetration or compression test parameters. Similar findings were reported by Brady and Penfield (1982) for samples heated as intact roasts.

Table 2—Effect of endpoint temperature on heating characteristics of beef roasts[a,b]

Parameter	Endpoint temperature		
	60°C	70°C	80°C
Heating time (min/kg)	69.01a	76.00ab	89.64b
Total losses (%)	14.93a	24.23b	23.75b
Drip loss (%)	1.85a	4.56b	4.50b
Evaporative loss (%)	13.08a	19.67b	19.25b
Moisture (%)	67.25a	65.04a	62.72a

[a] Means of 4 replications.
[b] Means in the same row followed by different letters are significantly different (p < 0.05).

Table 3—Effect of endpoint temperature on sensory tenderness characteristics of roast beef[a,b]

Sensory characteristic	Endpoint temperature		
	60°C	70°C	80°C
Springiness	5.21	5.00	5.65
Hardness	6.88	6.00	6.50
Deformation before rupture	9.80	8.67	8.67
Chewiness	7.84y	6.77x	6.69x
Strand separation	3.84	3.95	4.09
Mealiness	4.40b	8.03a	5.39b
Moisture	10.57b	8.03a	8.23a
Oiliness	3.31	3.24	3.06
Tenderness	9.14	9.96	9.42

[a] Means of 4 replications, 9 panelists/replication.
[b] Means in the same row followed by different letters are different (p < 0.05, x,y; p < 0.01, a-c).

Table 4—Effect of endpoint temperature on objective parameters of roast beef texture[a,b]

Objective parameter	Endpoint temperature		
	60°C	70°C	80°C
Shear			
Firmness (kg/min)	0.71b	0.48a	0.66b
Cohesiveness (kg)	8.67c	6.02a	7.16b
Penetration			
Hardness (kg)	1.46a	1.89b	2.27c
Compression			
Hardness (kg)	15.25a	24.59b	20.75b
Springiness (min)	4.18a	5.32b	5.23b
Cohesiveness	0.43a	0.42a	0.47a

[a] Means of 4 replications with 4 samples/replication.
[b] Means in the same row followed by different letters are different (p < 0.01).

Table 5—Correlations between sensory and instrumental measures of roast beef texture[a]

Sensory parameters	Shear		Penetration	Compression		
	Firmness	Cohesiveness	Hardness	Springiness	Hardness	Cohesiveness
Springiness	0.434	0.141	0.219	−0.087	0.344	−0.061
Hardness	0.653*	0.641*	−0.154	−0.216	0.112	0.201
Deformation	−0.614*	0.458	−0.097	−0.321	−0.196	0.074
Chewiness	0.650*	0.801**	−0.241	−0.398	−0.136	0.096
Separation	0.367	0.417	0.461	0.124	−0.054	0.492
Mealiness	−0.068	−0.250	0.205	0.341	0.597*	0.162
Moisture	−0.080	0.192	−0.412	−0.454	−0.681*	−0.261
Oiliness	−0.385	−0.427	−0.188	−0.050	−0.045	−0.707*
Tenderness	−0.668*	−0.688*	0.047	0.139	−0.054	−0.229

[a] n = 12
* $p < 0.05$; ** $p < 0.01$

Hardness, measured by compression, was associated positively with sensory scores for mealiness and negatively with those for moisture suggesting that this instrumental parameter was strongly influenced by the moisture content of the samples. Compression cohesiveness was negatively correlated with panel scores for oiliness. Sensory parameters of springiness and separation were not highly correlated with any of the instrumental parameters measured and the instrumental parameter penetration hardness was not related to any of the sensory parameters.

The strong correlations between the sensory characteristics of chewiness, hardness and tenderness would indicate that these parameters were measuring either the same element of tenderness or ones that were strongly related. Since the two shear parameters, firmness and cohesiveness, were both related to these sensory parameters apparently they also were measuring similar elements of texture and would be reliable in predicting sensory evaluations of these characteristics. Instron texture profile analysis was found to be related to panel evaluations of textural attributes only in the relationship of compression hardness to panel moisture and mealiness scores and compression cohesiveness with oiliness. The force to penetrate the samples with a blunt probe was not found to be related to any of the parameters evaluated by the panel.

Since both sensory and instrumental techniques are currently widely used to evaluate meat texture, it is important that the relationships between these types of tests be clearly defined. The relationships identified in this study provide a better understanding of the textural elements being evaluated by the various types of tests and provide a basis for relating measurements made by sensory panels and various instrumental test procedures. However, more work needs to be done in order to totally define the relationships between sensory and instrumental tests of meat texture.

REFERENCES

AOAC. 1980. "Official Methods of Analysis," 13 ed. Association of Official Analytical Chemists, Washington, DC.

Bourne, M.C. 1978. Texture profile analysis. Food Technol. 32(7): 62.

Bouton, P.E., Ford, A.L., Harris, P.V., and Ratcliff, D. 1975. Objective-subjective assessment of meat tenderness. J. Texture Studies 6: 315.

Bouton, P.E., Harris, P.V., and Ratcliff, D. 1981. Effect of cooking temperature and time on the shear properties of meat. J. Food Sci. 46: 1082.

Bouton, P.E., Harris, P.V., and Shorthose, W.R. 1971. Effect of ultimate pH upon the water-holding capacity and tenderness of mutton. J. Food Sci. 6: 315.

Brady, P.L. and Penfield, M.P. 1982. Effects of heating system, temperature and rate of heating on sensory characteristics of beef. J. Food Sci. 47: 1783.

Civille, G.M. and Szczesniak, A.S. 1973. Guidelines to training a texture profile panel. J. Texture Studies 4: 204.

Cover, S., Ritchey, S.J., and Hostetler, R.L. 1962. Tenderness of beef. I. The connective tissue component. J. Food Sci. 27: 469.

Cross, H.R., Stanfield, M.S., and Koch, E.J. 1976. Beef palatability as affected by cooking rate and final internal temperature. J. Anim. Sci. 43(1): 114.

Larmond, E. 1976. Sensory measurement of food texture. In "Rheology and Texture in Food Quality," p. 535. AVI Publishing Co., Westport, CT.

Larmond, E. and Petrasovits, A. 1972. Relationship between Warner-Bratzler and sensory determinations of beef tenderness by the method of paired comparisons. Can. Inst. Food Sci. Technol. J. 5(3): 138.

Martens, H., Stabursvik, E., and Martens, M. 1982. Texture and colour changes in meat during cooking related to thermal denaturation of muscle proteins. J. Texture Studies 13: 291.

Paul, P.C., McCrae, S.E., and Hofferber, L.M. 1973. Heat-induced changes in extractability of beef muscle collagen. J. Food Sci. 38: 66.

Rhodes, D.N., Jones, R.C.D., Chrystall, B.B., and Harries, J.M. 1972. Meat texture. 2. The relationship between subjective assessments and a compressive test on roast beef. J. Texture Studies 3: 298.

Szczesniak, A.S. 1963. Classification of textural characteristics. J. Food Sci. 28: 385.

Szczesniak, A.S. 1968. Correlations between objective and sensory texture measurements. Food Technol. 22: 981.

Growth of *Clostridium sporogenes* and *Staphylococcus aureus* at Different Temperatures in Cooked Corned Beef Made with Reduced Levels of Sodium Chloride

R. C. WHITING, R. C. BENEDICT, C. A. KUNSCH, and D. BLALOCK

──────── ABSTRACT ────────

Cooked corned beef made with normal (ca. 2.5%) or a reduced (ca. 1.5%) level of salt were inoculated with either clostridial spores or with staphylococci and incubated at temperatures ranging from 5 - 30°C. Growth of indigenous microflora, staphylococci, or clostridia was similar at both salt levels at a given incubation temperature. However, increasing the abuse temperature greatly increased the growth of all organisms. Outgrowth of clostridial spores occurred in ground cooked corned beef which contained the normal residual nitrite of 40 - 45 ppm; readdition of nitrite to 150 ppm at the time of inoculation markedly reduced growth. Gas production was not a good indicator of clostridial growth.

INTRODUCTION

REDUCING DIETARY SODIUM has been recommended to decrease the incidence of hypertension and subsequent occurrences of cardiovascular disease, stroke, renal failure, and decreased life span in individuals susceptible to these conditions (Pearson and Wolzak, 1982; Sebranek et al., 1983). Processed meat products are an important source of dietary sodium. For example, corned beef loaf contains an average of 1,037 mg sodium/100g (USDA, 1980). However, the sodium content within a class of meat products currently in the marketplace often ranges more than two-fold, e.g., the content in hams ranged from 654 - 2004 mg sodium/100g (Sebranek et al., 1983).

Sodium chloride has three functions in meat products: providing and enhancing flavor, solubilizing proteins to create desired texture, and controlling microbial growth to enhance shelf life and inhibit pathogens (Ingram and Kitchell, 1967; Terrell, 1983). The microbiological preservation and safety of most meat products is a result of a combination of salt and nitrite levels, pH, heat processing, vacuum packaging, and refrigeration. Few studies have described the changes in microbiological growth in semi-preserved meat products with reductions of 20 - 50% from current salt levels (Rieman et al., 1972; Smith et al., 1983). Terrell and Brown (1981) found that only a relatively high salt level (brine content above 4.5%) reduced the growth of aerobic bacteria in vacuum packaged frankfurters. Sofos (1983) reduced the salt content of frankfurters from 2.5% to 1.5% and observed that pH of the batter was more important than salt level in determining growth of psychrotropic and mesophilic bacteria. Kraft (1983) found that a similar salt reduction in bologna, bacon, or ham did not adversely affect shelf life. Greenberg et al. (1959) using a model ham product showed that *Clostridium botulinum* growth and toxin production were not inhibited at less than 9% brine. Toxin was formed when brine contents exceeded 6.25% even though the ham lacked signs of obvious spoilage. Whiting et al. (1984) found temperature to be a more important factor than salt in controlling growth of aerobic bacteria, facultative bacteria, and *Staphylococcus*

aureus in frankfurters. *Clostridium sporogenes* spore outgrowth was effectively inhibited, probably by the nitrite.

This study will determine the growth of bacteria in cooked and vacuum packaged corned beef made with a reduced level of sodium chloride. Growth of the organisms will be followed at various abuse temperatures.

MATERIALS & METHODS

Corned beef

Beef bottom round was obtained fresh from a local distributor. The meat was trimmed of excess surface fat and connective tissue and portions from the same postmortem muscles were distributed to the various curing treatments to minimize pH and compositional differences that were not a consequence of the curing itself. The manufacture of cooked corned beef followed the procedures given by Komarick et al. (1974). The curing pickle contained 0.16% $NaNO_3$, 0.16% $NaNO_2$, 0.44% sodium ascorbate, 2.4% sucrose, and 2.7% sodium tripolyphosphate. The sodium chloride content of the curing pickle was varied so that adding the pickle at 15% of the green weight would yield corned beef with the desired salt content. Approximately two-thirds of the pickle was injected into the meat with a single needle pumping system, and the remainder was added as a cover pickle. Bags containing a 3 - 6 kg piece of round plus pickle were vacuum sealed to have continuous contact of the meat and pickle and were cured at 6°C for 5 days.

After curing, the corned beef was cooked in the bag with the small amount of remaining brine by immersion in 80 - 90°C water until temperature probes inserted in the center of the piece indicated 71°C. The cooked corned beef was rapidly cooled in ice water, cut into approximately 1kg pieces, and frozen at −18°C until needed. Enough corned beef was made so that replicate runs of an experiment could be made from one batch to minimize variation in salt content.

Pieces of cooked corned beef were thawed at 1°C and sliced with a meat slicer. Twenty-five grams of slices were placed into vacuum pouches. They were then inoculated, vacuum sealed (0.97 bar), and stored at various temperatures.

Because of the difficulty in achieving an exact salt level after curing and cooking, portions of the cooked corned beef containing an average of 1.2% salt were ground uniformly, and additional salt and/or a sodium nitrite solution was added to achieve up to 4.0% salt and 150 ppm $NaNO_2$. After mixing and standing for several hours, 25g were weighed into pouches, inoculated with clostridia, vacuum sealed, heat shocked at 80°C for 10 min, and stored.

Chemical analyses

Water content of the cooked corned beef was determined by air drying procedures (AOAC, 1980). Sodium content was obtained by dry ashing at 525°C, dissolving in nitric acid, and measuring by atomic absorption spectroscopy (AOAC, 1980). Residual nitrite was estimated by the Griess reagent following the AOAC (1980) procedure except that sulfanilic acid and 1-naphthylamine were added simultaneously.

Microbiological methods

The microbiological techniques were similar to those used in an equivalent study of frankfurters (Whiting et al., 1984). Aerobic plate counts were made by homogenizing the entire contents of a pouch with 50 mL of 0.1% peptone water in a Stomacher 400 for 2 min. Three milliliters were taken and dilutions were made with peptone water; and 0.1 mL aliquot was spread on APT agar (Difco) and incubated at 20°C.

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A *S. aureus* 196E stock culture was grown with shaking in 250 mL Brain Heart Infusion (BHI) (Difco) at 37°C for 48 hr. Aliquots of 1 or 10 mL were placed into vials, frozen in dry-ice acetone, and stored at −13°C. Thawed samples plated on TSA agar (Difco) containing 7.0% additional salt (TSAS agar) had a viable count of 7×10^7 CFU/mL. Added salt inhibits most bacterial species, but this strain of *S. aureus* grows consistently as golden-colored colonies. To inoculate the corned beef, an aliquot of *S. aureus* stock culture was thawed and diluted so that 0.25 mL inoculated into the pouches would contain approximately 10^3 organisms/g.

After a predetermined storage time, the entire contents of a pouch were transferred to a Stomacher bag. Fifty milliliters of peptone water were added, part of the peptone water was used to rinse the pouch. Three milliliters were removed for diluting and plating, and the *S. aureus* were enumerated as golden colonies on TSAS agar after incubation at 37°C for 48 hr.

C. sporogenes (B1219) is a nontoxigenic anaerobic spore former similar go the proteolytic strains of *C. botulinum*. A spore suspension was prepared in beef heart infusion according to Santo-Goldoni et al. (1980). Spores were inoculated onto the cooked corned beef at 9.4×10^3 spores/g. The pouches were vacuum sealed, heat shocked at 80°C for 10 min, and stored. After the desired storage, 50 mL 0.1% peptone-0.05% thioglycolate were added and the entire contents were homogenized in the original pouch by the Stomacher. Three milliliters were taken and diluted with peptone-thioglycolate. Aliquots were pour plated on Botulinum Assay Medium (BAM) (Huhtanen, 1975) and incubated at 37°C for 2 days in an anaerobic chamber flushed with a N_2-H_2-CO_2 gas mixture. This permits growth of the few indigenous anaerobes that would survive heat shocking as well as the inoculated *C. sporogenes*. Therefore a corresponding uninoculated package was enumerated each time that an inoculated package was. Initial counts in the uninoculated packages were usually less than the minimum detectable level of 10^2 CFU/g. Whenever the counts in the uninoculated packages approached or equaled those in the inoculated, the storage trial was terminated because the identity of the counts would then be in doubt. In some experiments clostridia were inoculated onto ground corned beef, and growth was observed by gas production and swelling of the vacuum pouches. A corresponding set of uninoculated pouches were also observed.

RESULTS & DISCUSSION

Natural flora

Atomic absorption analyses showed that the initial two batches of corned beef contained 1.24 ± 0.02% (mean and standard error of the mean) and 2.14 ± 0.13% NaCl (1.7% and 3.1% brine, respectively). This was less than the desired 1.5% and 2.5%, but the higher salt level was within the probable range of current commercial products. More importantly, there was a 0.90% difference in salt levels between the two samples. Samples were stored at 5°C, 11°C, and 16°C. After 14 days, some of the pouches that had been stored at 5°C were transferred to 20°C or 27°C to simulate a likely pattern of abuse.

Initial counts of facultative microorganisms on the cooked corned beef were approximately 10^4 colony forming units (CFU) per gram (Fig. 1). Growth was highly dependent on temperature. The counts exceeded 10^6 CFU/g after 2 days at 16°C, 4 days at 11°C, and 7 days at 5°C. When the corned beef was placed at 20°C or 27°C the growth was immediately very rapid although probably limited because the counts were already at 10^7 per gram. There was no strong evidence for the salt levels affecting growth. The 1.24% salt corned beef consistently had slightly greater counts through the growth phase, but the difference was never greater than 0.7 log cycle. Similar conclusions were reached by Kraft (1983) who found a 25% reduction in the salt content of hams would be acceptable insofar as microbiological spoilage was concerned. Whiting et al. (1984) reported little effect of salt reductions from 2.4% to 1.6% in frankfurters on the growth of the natural aerobic or anaerobic flora.

Staphylococcus aureus

Another set of pouches containing corned beef from the same batch as above was inoculated with 1×10^4 CFU *S. aureus*/g and stored under the same set of time-temperature conditions (Fig. 1). The initial counts were approximately 3×10^3 CFU/g indicating good survival of the inoculated cells.

Growth was highly temperature dependent, the rate of growth at 16°C was very similar to the indigenous flora. At 11°C, the staphylococci growth rate was much less than the indigenous flora, and at 5°C the staphylococci did not grow. However, after 14 days of no growth at 5°C the staphylococci showed very rapid growth when the temperature was elevated to 20°C or 27°C.

The two salt levels did not appear to affect the growth at any temperature. The 1.24% salt corned beef had somewhat lower staphylococci counts after 11 days at 11°C than did the 2.14% salt corned beef; this may be sampling variation or it may reflect the poor competitive ability of the staphylococci under less favorable conditions (Smith et al., 1983). The slowing of growth on the 1.24% salt corned beef during the second day at 20°C or 27°C may also reflect this competition or the beginning of the stationary growth phase. This inability of staphylococci to compete and grow more rapidly than the normal flora was also observed by Whiting et al. (1984) on reduced-salt frankfurters.

Clostridium sporogenes

Another set of cooked corned beef was prepared which contained 1.6 ± 0.2% and 2.4 ± 0.1% salt (2.4% and 3.6%
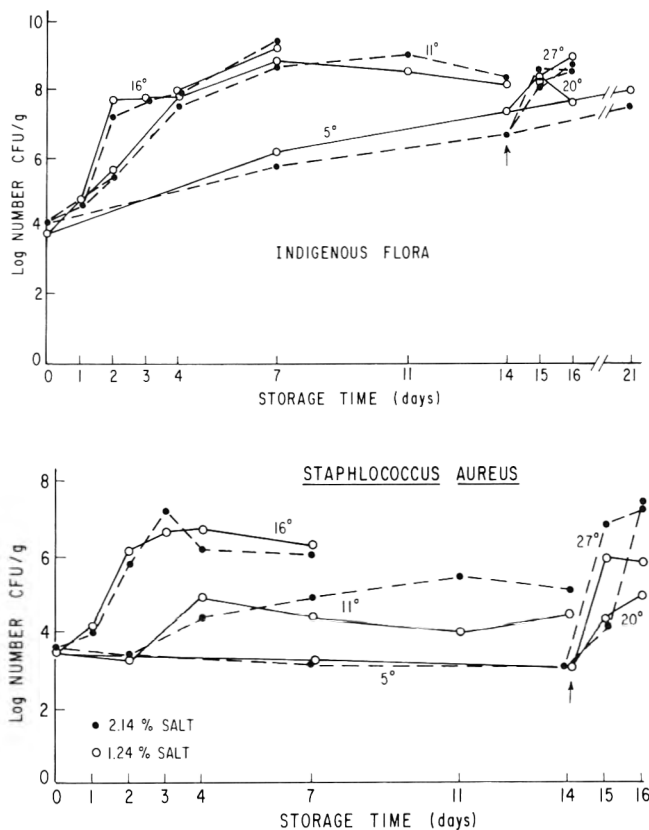
Fig. 1—Growth of indigenous facultative organisms and Staphylococcus aureus in cooked and vacuum packaged corned beef stored at various temperatures. Some packages were stored at 5°C for 14 days, and then shifted (indicated by ↑) to 20°C or 27°C for 1 or 2 days. Values are averages of two runs.

brine, respectively) and had average residual nitrite levels of 57 ± 2 ppm and 39 ± 1 ppm, respectively. Some of these pouches were inoculated with 10^4 spores of *C. sporogenes*/ g. All pouches were vacuum packaged, heat shocked, and stored at 16°C, 11°C, and 5°C for up to 14 days. After 9 days, designated pouches stored at 5°C were transferred to 30°C. Corresponding uninoculated samples were also enumerated on BAM media and their counts were always at least 2 log cycles less.

The growth of *C. sporogenes* was slower than the other organisms but the same pattern reappeared (Fig. 2). Growth was moderate at 16°C, slow at 11°C, and inhibited at 5°C. Raising the temperature to 30°C after 9 days at 5°C resulted in an extremely rapid growth. The growth on the 1.6% salt corned beef was slightly greater at 11°C and 16°C.

This growth on corned beef contrasted with the absence of growth in frankfurters (Whiting et al., 1984). A likely explanation is the lower residual nitrite levels in the cured and cooked corned beef when the spores were inoculated, compared to conditions in the frankfurter batter where spores were added soon after emulsifying (Holley, 1981; Hauschild, 1982; Pierson and Smoot, 1982; Robinson et al., 1982).

The relationship between clostridial growth and salt concentration was further examined by experiments in which cooked corned beef containing 1.2 ± 0.1% salt and 72.5 ± 1.2% water was ground and additional salt was added to portions to give a precisely defined series of salt levels. The salt concentration of 1.2%, 1.8%, 2.5%, 3.2%, and 4.0% had calculated brine concentrations of 1.6%, 2.4%, 3.3%, 4.2%, and 5.2%, respectively. Pouches were inoculated with 1.4 X 10^2 or 1.4 X 10^4 spores/g, heat shocked, and stored at 20°C for up to 60 days. A complete set of uninoculated pouches of corned beef were also heat shocked and stored. The appearance of gas and pouch swelling is shown on Fig. 3, the ordinate is the percentage of pouches with definite presence of gas although not necessarily enough for swelling. At 4% salt only a few small gas bubbles were produced that disappeared with continued storage. With the lower inoculation level, gas production was inconsistent at 4.0% salt; no gas production was observed during the second run. No gas appeared in the corresponding uninoculated packages except at a slow rate in the 1.2% salt samples.
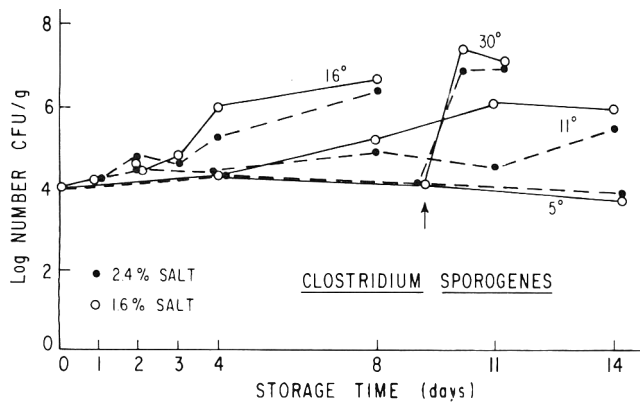
The larger inoculation caused a more rapid appearance of gas at all salt levels. This influence of inoculation size has been commented on by Riemann et al. (1972), Holley (1981), and Pierson and Smoot (1982). The inhibitory effect of salt appeared to become a significant factor at and above 3.2% salt, levels above many cured meat products. It is widely known that inhibition of growth and toxin production by proteolytic *C. botulinum* by salt alone requires at least 8.5% salt (Rieman et al., 1972; Pierson and Smoot, 1982).

The influence of nitrite was determined with a similar experiment. From corned beef with 1.3 ± 0.4% salt, lots containing 1.5%, 2.5%, and 3.5% salt were prepared, all with a residual 45 ppm nitrite. Calculated brine concentrations were 2.4%, 3.6%, and 5.4%. To some of the pouches with 1.5% and 2.5% salt, nitrite solutions were added to make a total of 150 ppm sodium nitrite. The pH of these samples averaged 6.04 ± 0.04. Most of the inoculated pouches with 1.5% and 2.5% salt and residual nitrite showed gas after 1 and 2 days at 20°C (Fig. 4). With additional nitrite this was delayed to 10 and 25 days, respectively. The 3.5% salt-45 ppm nitrite showed only trace of gas in a few packages after 35 days at 20°C. Gas production in the corresponding uninoculated pouches appeared much later than in the inoculated pouches in all of the salt-nitrite treatments.

Montville (1983) reported that growth and toxin production by *C. botulinum* can occur in media while gas production may be delayed or absent. Therefore, in the final experiment clostridial growth was also determined. Batches of ground corned beef contained 1.6 ± 0.2% and 2.4 ± 0.1% NaCl with residual 42 ± 1 ppm nitrite (2.4 and 3.6% brine, respectively). Supplemental salt to make 3.5% was added to the latter batch (5.6% brine). Portions of the 1.5% and 2.5% NaCl batches had sodium nitrite added to give 150 ± 10 ppm. Uninoculated controls were also run and a particular treatment was stopped when the counts in the uninoculated pouches equalled those in the inoculated. Gas production and growth were followed during incubation at 20°C.

Fig. 2—Growth of anaerobic bacteria in cooked and vacuum packaged corned beef inoculated with 10^4 spores/g Clostridium sporogenes and stored at various temperatures. Some packages were stored at 5°C for 9 days, then stored at 30°C for 1 or 2 days (indicated by ↑). Plotting was stopped when the number of colonies/g in the uninoculated packages equaled the number in inoculated packages. Values are averages of two runs.

Fig. 3—Presence of gas in vacuum packages of cooked corned beef inoculated with C. sporogenes and stored at 20°C. The corned beef contained from 1.2% to 4.0% salt and was inoculated with either 1.4 X 10^2 or 1.4 X 10^4 spores/g. Values indicate the percentage of packages containing gas and represent two runs of five packages per treatment.
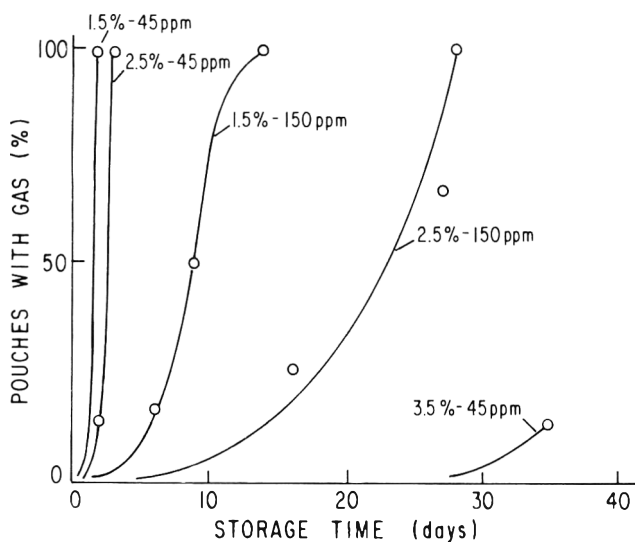
Fig. 4—Presence of gas in vacuum packages of cooked corned beef inoculated with C. sporogenes. The corned beef contained 1.5%, 2.5%, or 3.5% salt and residual nitrite of 45 ppm or added nitrite to 150 ppm. Inoculation was 1.3 X 10⁴ spores/g and packages were stored at 20°C. Values indicate the percentage of packages containing gas. Each treatment contained seven packages.

Fig. 5—Growth and gas production in vacuum packages of cooked corned beef inoculated with C. sporogenes. The corned beef contained various salt and nitrite levels. Storage was at 20°C. The time when half of the packages in a treatment contained gas is indicated by "G." Plotting was stopped when the number of CFU/g in the uninoculated packages equaled the number in the inoculated packages.

The three treatments containing only residual nitrite showed a rapid 2 – 3 log cycle increase in cell numbers that was moderately affected by salt level (Fig. 5). Gas appeared quickly in the corned beef containing 1.5% and 2.5% salt but had not appeared in the 3.5% salt samples at 13 days when this treatment was terminated. Corned beef with added nitrite had growth delayed approximately 3 days but cell populations exceeded 10^6 CFU/g after 6 days. The growth curves for 1.5% and 2.5% salt corned beef with 150 ppm nitrite were nearly identical during the growth phase; however, gas production occurred in a majority of the 1.5% salt-150 ppm nitrite sample after 9 days, but never occurred with 2.5% salt-150 ppm nitrite.

CONCLUSIONS

THESE RESULTS SHOWED that the temperature of storage or abuse was more important in determining the growth of indigenous microorganisms, S. aureus, and C. sporogenes than a reasonable salt reduction from the current industry averages. With refrigeration at 11°C, the normal flora outgrew both staphylococci and clostridia, even though large inocula were used in these studies.

The importance of considering nitrite levels with clostridial growth was apparent; reduction of both salt and nitrite from the current practice would increase the risks of clostridial growth. Clostridial growth was more vigorous when residual nitrite only was present. These results are particularly applicable to contamination occurring after curing and cooking. Further work is in progress on survival and growth of spores during the curing process.

This work confirmed observations that gas production can be a very misleading indicator of clostridial growth. When conditions become less favorable (higher salt and nitrite levels), gas production was inhibited much more than actual growth. This implied that a complete evaluation of the risk of botulism in reduced-salt meat products should not rely on gas production alone, but should include enumeration and toxin assays with C. botulinum.

REFERENCES

AOAC. 1980. "Official Methods of Analysis," 13th ed. Association of Official Analytical Chemists, Washington, DC.

Greenberg, R.A., Silliker, J.H., and Fatta, L.D. 1959. The influence of sodium chloride on toxin production and organoleptic breakdown in perishable cured meat inoculated with Clostridium botulinum. Food Technol. 13: 509.
Hauschild, A.H.W. 1982. Assessment of botulism hazards from cured meats. Food Technol. 36: 95.
Holley, R.A. 1981. Review of the potential hazard from botulism in cured meat. Can. Inst. Food Sci. Technol. J. 14: 183.
Huhtanen, O.N. 1975. Some observations on a Perigo-type inhibition of Clostridium botulinum in a simplified medium. J. Milk Food Technol. 38: 762.
Ingram, M. and Kitchell, A.G. 1967. Salt as a preservative for foods. J. Food Technol. 2: 1.
Kraft, A.A. 1983. Microbiology of low salt meat products. Proceedings, Meat Processing Conf., p. 10. Western States Meat Assn., San Francisco, CA.
Komarick, S.L., Tressler, D.K., and Long, L. 1974. "Food Products Formulary." AVI Pub. Co., Westport, CT.
Montville, T.J. 1983. Dependence of Clostridium botulinum gas and protease production on culture conditions. Appl. Environ. Microbiol. 45: 571.
Pearson, A.M. and Wolzak, A.M. 1982. Salt — Its use in animal products — A human health dilemma. J. Anim. Sci. 54: 1263.
Pierson, M.D. and Smoot, L.A. 1982. Nitrite, nitrite alternatives, and the control of Clostridium botulinum in cured meats. CRC Crit. Rev. Food Sci. Nutrit. 17: 141.
Riemann, H., Lee, W.H., and Genigeorgis, C. 1972. Control of Clostridium botulinum and Staphylococcus aureus in semi-preserved meat products. J. Milk Food Technol. 35: 514.
Robinson, A., Gibson, A.M., and Roberts, T.A. 1982. Factors controlling the growth of Clostridium botulinum types A and B in pasteurized, cured meats. V. Prediction of toxin production: Nonlinear effects of storage temperature and salt concentration. J. Food Technol. 17: 727.
Santo-Goldoni, J., Kojima, S., Leonard, S., and Heil, J.R. 1980. Growing spores of P.A. 3679 in formulations of beef heart infusion broth. J. Food Sci. 45: 467.
Sebranek, J.G., Olson, D.G., Whiting, R.C., Benedict, R.C., Rust, R.E., Kraft, A.A., and Woychik, J.H. 1983. Physiological role of dietary sodium in human health and implications of sodium reduction in muscle foods. Food Technol. 37: 51.
Smith, J.L., Buchanan, R.L., and Palumbo, S.A. 1983. Effect of food environment on staphylococcal enterotoxin synthesis: A review. J. Food Prot. 46: 545.
Sofos, J.N. 1983. Effects of reduced salt (NaCl) levels on the stability of frankfurters. J. Food Sci. 48: 1684.
Terrell, R.N. 1983. Reducing the sodium content of processed meats. Food Technol. 37: 66.
Terrell, R.N. and Brown, J.A' 1981. Salt, water, and oilseed proteins affect brine content of sausages. J. Food Prot. 44: 43.
USDA. 1980. "Composition of Foods," Agriculture Handbook No. 8-7. U.S. Government Printing Office, Washington, DC.
Whiting, R.C., Benedict, R.C., Kunsch, C.A., and Woychik, J.H. 1984. Effect of sodium chloride levels in frankfurters on the growth of Clostridium sporogenes and Staphylococcus aureus. J. Food Sci. 49(2): 351.

Characteristics of Low-Salt Frankfurters Produced with Enzyme-Modified Mechanically Deboned Fowl

DENISE M. SMITH and CLARK J. BREKKE

ABSTRACT

Objective and sensory methods were used to compare low-salt frankfurters containing enzyme-modified mechanically deboned fowl (MDF) with normal-salt (2%) and low-salt (0.5%) MDF frankfurter controls, a low-salt MDF pH control and a commercial chicken frankfurter. MDF was modified with Milezyme AFP 2000. There was no significant difference (P < 0.05) in reheated yield between the enzyme-modified treatment, the normal-salt control, and the commercial frankfurter. Enzymatic modification resulted in improved texture and had no adverse effect on cured meat color or flavor when compared to other low-salt treatments. Microbial numbers increased faster on the enzyme-modified low-salt frankfurter than on the normal-salt control.

INTRODUCTION

NaCl (salt) is generally added to processed red meat products at a concentration of 2.25 – 2.75% of the formulation (Olson and Terrell, 1981), although poultry frankfurters usually contain from 2.0 – 2.2% NaCl (Cook, 1980). Salt contributes to flavor, influences shelf life, and affects the functional responses of the myofibrillar proteins in a processed meat product (Olson, 1982). Functional roles of the myofibrillar proteins include fat bindng, water holding capacity, texture, and product yield (Acton et al., 1983).

A reduction in sodium consumption may lower hypertension in individuals genetically predisposed to the disease (IFT, 1980). Consequently, many meat processors are trying to reduce the sodium content in their processed products (Anon., 1983). Several methods have been investigated to reduce sodium in processed meat products and at the same time maintain quality, including reduced salt content (Sofos, 1983), replacing NaCl with other chloride salts (Hand et al., 1982; Olson and Terrell, 1981), salt preblending, use of pre-rigor meats (Puolanne and Terrell, 1983a) and use of phosphates and other additives (Puolanne and Terrell, 1983b).

Enzymatic modification has been used extensively to improve the functinality of low-function proteins and to tailor the functionality of certain proteins to meet specific processing needs (Richardson, 1977). Enzymatic modification of beef heart (Smith and Brekke, 1984) and mechanically deboned fowl (MDF) (Smith and Brekke, 1985) myofibrillar proteins improved protein functionality in model systems at both low and normal salt contents. Improvement in myofibrillar protein functionality at low salt levels suggested enzymatic modification as a partial alternative to salt when used in the production of processed meat products.

Model system results do not necessarily predict protein performance in processed products. The objective of this research was to incorporate enzymatically modified MDF into a low-salt frankfurter prepared on a pilot plant scale and to compare this product with MDF frankfurter controls produced with normal and low levels of salt and to a commercial chicken frankfurter.

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MATERIALS & METHODS

Materials

A 23 kg block of frozen mechanically deboned fowl (MDF) was purchased from Tony Downs Foods Co. (St. James, MN). The MDF was cut into 1 kg blocks, wrapped in polyethylene, and stored at –30°C. Pork back fat was obtained from the Washington State University Meat Laboratory (Pullman, WA). The pork fat was ground two times through a 6 mm plate and stored in polyethylene bags at –30°C. MDF and fat were analyzed for moisture, protein, and fat (AOAC, 1980). Milezyme AFP 2000, containing 2000 Spectrophotometric Acid Protease Units/mg protein, was obtained from Miles Laboratories (Elkhart, IN). Chicken frankfurters, Bar-S Tasty Dogs (Bar-S Foods Co., Phoenix, AZ), were purchased locally.

Preparation of enzyme-modified fowl

MDF equilibrated to 20°C was placed in a Kitchen Aid Stand Mixer (Model K5-A, Hobart Corp., Troy, OH) and the pH reduced to 3.2 with 3N HCl while mixing with the paddle attachment at the lowest speed setting. Hydrolysis was initiated by adding 1% AFP 2000, based on the protein content of the meat. The meat was mixed for 1 min and thereafter for 30 sec at 10 min intervals. The enzyme was inactivated after the desired time interval by adjusting the pH to 7.0 with 3N KOH. Smith and Brekke (1985) found AFP 2000 completely inhibited at pH 7.0. Hydrolysis was followed by measuring the increase in protein solubility with time of enzyme treatment as described by Smith and Brekke (1985). A pH control was prepared following the same procedure, except that enzyme was not added. Modified MDF was stored at 4°C until used the next day.

Frankfurter preparation

Frankfurter treatments tested were normal and low-salt (NaCl) MDF controls, a low-salt MDF pH control, and an enzyme-modified low-salt MDF sample. Pork fat and MDF were thawed overnight at 4°C. Both the pH control and the enzyme-modified frankfurters were prepared as a 50:50 blend (based on the protein content of the MDF) of modified and control MDF. MDF was modified with Milenzyme AFP 2000 to produce a protein solubility of 38% in 0.1M NaCl, pH 7.0. This extent of MDF modification was chosen as it produced maximum cooked yields in meat model system experiments (Smith and Brekke, 1985). Ingredients were weighed out according to each treatment formulation (Table 1) and the fat and MDF held another 24 hr at 4°C for complete tempering. Formulations were designed to give identical proximate compositions for all treatments.

Frankfurters were manufactured in a 12°C processing room. MDF, spice mix (including sodium nitrite and ascorbic acid), salt, half the water, and enzyme-modified MDF, if called for in the formulation, were placed in a Hobart silent cutter (Model 94142, Troy, OH). The ingredients were chopped for 5 min (1725 rpm) until the batter temperature reached 6 – 9°C. The fat and remaining water were added, and the ingredients chopped for 5 min until the batter temperature reached 11 – 12°C.

Immediately after chopping, the batter was stuffed into 25 mm Nojax casings (Union Carbide, Chicago, IL) using a Frey 20 liter Electro-Hydraulic Stuffer (Koch Supplies, Inc., Kansas City, MO). Frankfurters were linked into 13 cm lengths using a Koch manual linking machine (Kansas City, MO) and each batch weighed separately. Frankfurters were hung on a rack and held until all formulations were prepared (ca. 1 hr). The unprocessed frankfurters were rinsed with a cold water spray and placed in the smokehouse (Enviro-Pak, Portland, OR).

Frankfurters were processed according to the smokehouse schedule shown in Table 2. When the frankfurter internal temperature reached 69°C the smokehouse was turned off and the frankfur-

ters cooled in a cold water spray until the internal temperature reached 32°C (ca. 5 min). Frankfurters were held in a 4°C cooler overnight and reweighed to determine smokehouse/storage yield. Duplicate batches of each treatment were prepared.

Frankfurter casings were removed by hand and frankfurters stored in polyethylene bags at 4°C. Three frankfurters from each batch were analyzed for moisture, protein, fat, and salt following AOAC procedures (1980).

Frankfurter sensory evaluation

Frankfurters were evaluated for chicken frankfurter flavor, chewiness, firmness, and frankfurter pink color. Twenty panelists, composed of 16 males and 4 females, aged 20 to 56, evaluated the frankfurters. Selection of the judges, who were students, faculty or staff, was based on availability and interest. One training session was held to familiarize the panelists with the evaluation procedures. Textural characteristics were explained and demonstrated by using commercial and pilot plant samples of frankfurters which exhibited a range of the sensory properties being evaluated.

Testing of flavor and texture was conducted in partitioned booths in the Dept. of Food Science & Human Nutrition Sensory Evaluation Laboratory. Red lights were used to minimize color bias during flavor and texture tests. Tasting sessions were held on one day. Flavor was evaluated at 10 a.m. and texture and color at 2 p.m. Frankfurters were served in covered beakers coded with random three-digit numbers and presented in a balanced block design. Panelists were instructed to expectorate after tasting and rinse their mouths with room temperature distilled water between samples. Frankfurters from each treatment were sliced into 2 cm lengths. One piece of each treatment sample and two pieces of the reference sample were each placed in a 50 mL beaker containing 4 mL of distilled water and covered with aluminum foil. The beakers were placed in pans of hot water and heated in a 177°C oven for 20 min to an internal temperature of 42°C. Reheated frankfurters were held on the open door of a 93°C oven until served in pans of warm water to minimize cooling.

Color was evaluated by placing the nonreheated reference and treatment frankfurters on a white background under a fluorescent light which simulated C.I.E. illuminant C brightness. Frankfurters were coded with a random three-digit number. Both the skin and internal color of a frankfurter which had been sliced lengthwise were evaluated. An unstructured 100 mm scale with anchor points 5 mm from each end was used to evaluate the frankfurters. The ends of the scale were labeled "Less" and "More" with the center labeled "Same as reference". Panelists were asked to determine the amount each sample deviated from the reference (high-salt control frankfurter). A hidden reference was included as a test sample to check internal variation of the judges' response. Five treatments were evaluated in duplicate, so that panelists received 10 samples plus the reference each session.

Results were analyzed by analysis of variance. Main effects tested were treatments, judges, replications, and reference replication, as well as two-way interactions. Duncan's new multiple range test was used to calculate significant differences between treatments (Steel and Torrie, 1960).

Microbiological stability

Microbiological stability of the frankfurters was monitored by surface aerobic plate counts over a 25-day storage period (Kotula et al., 1980). The day following manufacture and processing, casings were removed and the frankfurters were placed as aseptically as possible in sterile Whirlpak® bags, one to a bag, and stored at 4°C.

Two frankfurters from each treatment were separately weighed and each was transferred to a canning jar containing 450 mL sterile phosphate-MgCl diluent (2 mM $MgCl_2$, 0.3 mM K-phosphate, pH 7.2). The jars were shaken in a standard arc (Clark et al., 1978) and, after appropriate dilutions, pour plates were made with Standard Plate Count agar (Difco Laboratories, Detroit, MI). The plates were incubated at 20°C for 72 hr. Dilutions were calculated based on the weight of the frankfurters and volume of diluent. Aerobic plate counts of the MDF and the raw frankfurter emulsions were determined after incubation of the plates at 20°C for 72 hr using Standard Plate Count agar. The raw frankfurter emulsions were obtained prior to stuffing, placed in sterile Whirlpak® bags, and held at 4°C for 4 hr before plating.

Objective color measurement

A Hunter Lab Digital Color and Color Difference Meter, Model D25D (Fairfax, VA), was used to evaluate frankfurter internal color. The instrument was standardized with the pink standard plate, No. 25 W 824, with color values of L = 73.0, a = 10.8, and b = 5.7. Two pieces of black electrical tape were placed parallel to each other, at a distance of 17 mm, across the specimen port so the frankfurters were evaluated as a single specimen. Sample frankfurters were cut in half lengthwise, then cut into 3.5 cm sections. The internal surface was placed on the specimen port, and L and a_L values read immediately. Within each treatment, two sections from each of the three frankfurters were evaluated.

Severe reheat weight change

A frankfurter was weighed and placed in 400 mL boiling water for 10 min. The frankfurter was removed from the cooking water, cooled 5 min at room temperature, blotted dry, and reweighed to calculate a severe reheat cooked yield. The cooking water was transferred to a graduated cylinder and allowed to cool to room temperature. The volume of fat in the cooking water was recorded. Three frankfurters from each treatment were tested.

Texture evaluation

A Fudoh Rheometer (Fudokogyo Co., LTD, Tokyo, Japan) was used to measure compression rupture force (chewiness) and compression slope (firmness) as described by Smith and Brekke (1985). Frankfurters were prepared for skin strength measurements by cutting them crosswise into 2.5 cm lengths. The piano wire attachment for measuring skin strength consisted of a 3.2 cm length of wire (27 gauge, 0.355 mm in diameter) strung between two parallel supports. The frankfurter piece was laid horizontally and compressed by the rheometer between a flat surface and the piano wire under a crosshead speed of 6 cm/min to 19.6 N force.

Table 2—Smokehouse schedule for processing mechanically deboned fowl frankfurters

Smokehouse temp (°C)	Time (min)	Relative humidity (%)	Smoke
54	30	*	no
63	30	**	yes
77	ca. 100	39	yes

*Dampers open
**Dampers closed

Table 1—Weight of ingredients used in MDF frankfurter formulations

Treatment	Untreated MDF (g)	Treated MDF (g)	Fat (g)	Water (mL)	Spice mix[a] (g)	Salt (g)	Total (g)
Control, normal-salt	2245	0	439	297	55	61.6	3907.6
Control, low-salt	2245	0	439	297	55	21.2	3057.2
pH Control, low-salt	1050	1121	416	239	51	14.5	2891.5
Enzyme-modified, low-salt	1112	1205	438	188	54	15.1	3031.1

[a] Spice mix composition: sugar, 33.5g; white pepper, 9.2g; ground coriander, 5.9g; ground nutmeg, 3.9g; garlic powder, 0.8g; sodium nitrite, 0.4g; ascorbic acid, 1.3g.

The force required for the wire to break through the frankfurter skin was defined as the skin strength and indicated as a sudden decrease in the slope of the force-time curve.

Statistics

All objective test results were tested for significance (P < 0.05) using Duncan's new multiple range test (Steel and Torrie, 1960).

RESULTS & DISCUSSION

AFP 2000, AN ACID PROTEASE, has optimal activity at pH 3 and is inhibited by adjusting the pH to 7.0 or above. Adjustment of pH did not have an adverse effect on the functionality of isolated myofibrillar proteins when tested in model systems (Smith and Brekke, 1985). By choosing an acid protease, the use of heat or chemical inhibitors to inhibit enzyme activity was avoided.

Several preliminary experiments were conducted to establish procedures and formulations. Although the proximate composition of the MDF was 65.9% moisture, 14.4% protein, and 18.3% fat, additional fat was added to stress the emulsification properties of the meat proteins in the formulation. Frankfurters were formulated to contain 10.5% protein, 26.0% fat and 59.7% water. A stable, normal-salt control frankfurter could not be produced in the pilot plant when greater than 26% fat was used in the formulation. Ingoing salt concentrations were 2.0% for the normal-salt control and 0.5% for the low-salt frankfurters. It was found that 0.5% salt was the least amount which could be added, yet still allow for ease in casing removal and production of a satisfactory frankfurter.

Proximate composition

The proximate composition of the MDF frankfurters and a commercial chicken frankfurter was determined (Table 3). The commercial frankfurters were lower in protein and fat, and higher in moisture than the test frankfurters. There were no significant differences in proximate composition among the treatments, except the normal-salt control contained significantly less protein than the enzyme-modified sample.

The normal-salt control and the commercial frankfurter both contained 2.3% salt. All the low-salt frankfurters contained 0.7% salt, as the amount of salt produced during acid and base addition to the pH control and the enzyme-modified sample was calculated in the formulation. The pH of all frankfurters ranged from 6.3 – 6.5. Raising the pH of half the added MDF to 7.0 did not have any appreciable effect on the final product pH.

Product yields

Smokehouse/storage yields of the frankfurter treatments are shown in Table 4. There were no significant differences among smokehouse/storage yields except the pH control had a significantly lower yield than the enzyme-modified treatment. It appears that acid addition had a detrimental effect on meat protein functionality, possibly due to denaturation of sarcoplasmic proteins by acid. This was previously indicated in emulsified meat system experiments (Smith and Brekke, 1985).

The smokehouse/storage yields in this study were less than smokehouse yields alone reported for commercial frankfurter operations, where an 8 – 10% weight loss in the smokehouse is considered typical for frankfurters (Kramlich, 1971). Smokehouse yield is affected by both the nature of the product and the relative humidity/temperature combinations used during processing (Forrest et al., 1975). Either one of these factors may have contributed to the low smokehouse/storage yields obtained. Also, some of the observed weight loss could have resulted from storing frankfurters overnight before reweighing.

There was no significant difference in severe reheat yield between the enzyme-modified low-salt frankfurter, the commercial frankfurter, or the normal-salt control (Table 4). The low-salt control had the lowest yield, followed by the pH control. The low-salt control and the pH control exhibited visual shrinkage after severe reheat treatment. The volume of fat in the cook-out liquid decreased as the severe reheat yield increased among the frankfurters (Table 4).

During pH adjustment, 0.3% salt is added to the meat as acid and base, and the meat undergoes some blending. Improved severe reheat yields for the pH control may be a result of this procedure, since pre-blending with reduced salt is an effective way to lower salt concentrations in frankfurters, yet maintain yields (Olson, 1982). However, this explanation cannot explain the decrease in smokehouse yield of the pH control treatment.

Microbiological examination

The raw MDF contained 1.3×10^6 organisms/g as determined by aerobic plate count. Microbial counts for the raw frankfurter emulsions were not appreciably greater than for the raw MDF (Table 5). The pH control and enzyme-modified MDF were prepared at room temperature, which did not cause greater than normal increases in microbial num-

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Table 3—Proximate composition of chicken frankfurters after smokehouse processing[a]

Treatment	Protein (%)	Fat (%)	Moisture (%)	Salt (%)	pH
Control, normal-salt	12.8[b]	26.7[b]	54.6[b]	2.35[b]	6.4
Control, low-salt	13.1[b,c]	27.1[b]	55.3[b]	0.72[c]	6.4
pH Control, low-salt	13.3[b,c]	27.8[b]	54.3[b]	0.75[c]	6.5
Enzyme-modified, low-salt	13.5[c]	27.7[b]	54.3[b]	0.72[c]	6.5
Commercial	12.5[b]	23.5[c]	56.9[b]	2.34[b]	6.3

[a] Values are the average of triplicate determinations from duplicate batches.
[b,c] Means in the same column followed by the same letter did not differ significantly (P < 0.05).

Table 4—Product yields for chicken frankfurters

Treatment	Smokehouse/ Storage Yield[a] (%)	Severe Reheat Yield[b] (%)	Volume of Cook-Out Fat (ml)
Control, normal-salt	84.4[c,d]	98.8[c]	1.0
Control, low-salt	84.6[c,d]	74.1[d]	7.0
pH Control, low-salt	80.6[d]	87.3[e]	4.0
Enzyme-modified, low-salt	84.9[c]	97.1[c]	1.0
Commercial	NA	100.9[c]	1.0

[a] Values are the average of duplicate batches. NA = not available.
[b] Values are the average of triplicate determinations from each of two batches.
[c,d,e] Means followed by the same letter did not differ significantly (P < 0.05).

Table 5—Total aerobic counts of raw frankfurter emulsions[a]

Treatment	Organisms/g
Control, normal-salt	9.8×10^5[b]
Control, low-salt	1.9×10^6[b]
pH Control, low salt	1.5×10^6[b]
Enzyme-modified, low-salt	1.3×10^6[b]

[a] Values are the average of two determinations from each emulsion.
[b] Means in the same column followed by the same letter did not differ significantly (P < 0.05).

bers in the raw frankfurter emulsions, as the meat was at pH 3.2 for most of the time it was at room temperature. Common meat microorganisms do not grow at pH 3.2 (Ayres et al., 1980).

Surface aerobic plate counts of the MDF frankfurters were examined during a 25-day storage period (Fig. 1). Surface counts were less than 300 organisms/g one day after processing to an internal temperature of 69°C. All the low-salt frankfurters increased in microbial numbers at a faster initial rate than the normal-salt control during the storage period, although the only significant difference occurred between the enzyme-modified low-salt frankfurter and the normal-salt control. The preparation procedures were not responsible, since the raw emulsion counts for the enzyme-modified sample were not elevated. It is possible that proteolysis during enzymatic modification may have increased nutrient bioavailability. Sofos (1983) reported only a slight, and not significant, difference in microbial growth among frankfurters produced with various salt concentrations when stored vacuum packaged at 8°C for 31 days.

Reducing the salt content will reduce the lag time of bacterial growth, but very little research has been done in this area (Olson, 1982). Whiting et al. (1984) reported that frankfurters prepared with 1.5% salt had aerobic counts almost 1 log cycle greater than frankfurters made with 2.5% salt during an 11 day storage period. Reducing salt by 50% in ground pork inoculated with certain microbes had no effect on the growth of *Micrococcus* and *Moraxella* species, but *Lactobacillus* populations were increased (Terrell et al., 1983). Since commercial frankfurters are vacuum packaged, an additional study utilizing vacuum packaged MDF frankfurters is necessary.

Validity of the sensory evaluation experiment

Statistical analysis of the sensory data indicated significant differences (P < 0.05) between treatments, but not between replications or references (Fig. 2). Significant differences (P < 0.05) occurred among judges, except for the color evaluation. Differences among judges were probably due to the brief training period. Interaction of treatment by judges was not significant, indicating that variability in the judges' responses did not influence variability in treatments.
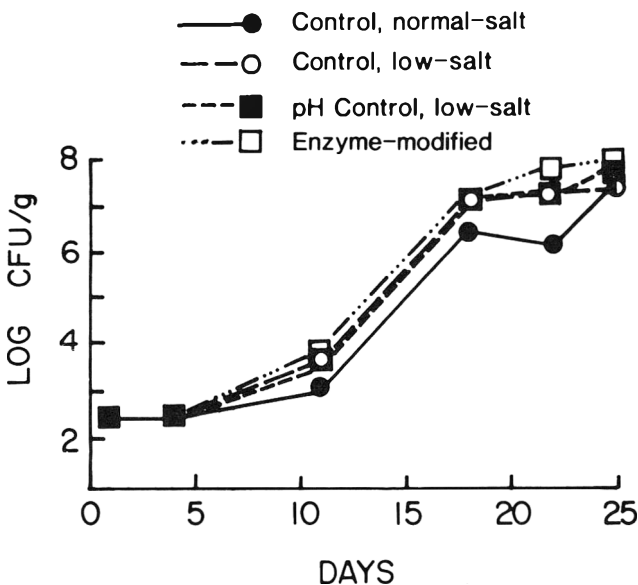
Frankfurter color

Frankfurters prepared from chicken muscle generally exhibit a pale cured meat color unless certain additives or special procedures and carcass parts are used, due to low myoglobin content of chicken meat. Mechanically deboned chicken contains about 0.06 μmols myoglobin/g of meat on a wet weight basis (Lee et al., 1975), which is much lower than the myoglobin content of beef (Lawrie, 1979).

All the experimental frankfurters were lighter and less red in internal color than the commercial chicken frankfurter (Table 6). The commercial frankfurter contained paprika and oleoresin of paprika to intensify the color. The pH control and enzyme-modified frankfurters were less red than the low- and normal-salt controls, for both the non-reheated and reheated samples, although differences were not significant for the reheated frankfurters (Table 6). The incorporation of air during mixing of the meat and partial denaturation of myoglobin with acid during preparation of the enzymatically modified MDF facilitates the conversion of myoglobin to metmyoglobin (Forrest et al., 1975). This may have been detrimental to cured meat color formation, as myoglobin must be in a reduced state to form the nitrosohemochromogen pigment (Forrest et al., 1975). Ascorbic acid, included in the frankfurter formulation, helps prevent this occurrence under normal processing procedures, but the conversion to metmyoglobin probably occurred in the modification procedure, before the addition of the ascorbic acid.

All MDF frankfurters were scored significantly lower in typical pink color than the commercial frankfurter by the sensory panel (Fig. 2). The normal-salt control and the enzyme-modified frankfurter had a more typical pink color than did the pH control and the low-salt control, according to the sensory panelists. The sensory panel did not agree entirely with the objective measurements of internal frankfurter color. There are several possible explanations for this discrepancy in results between the sensory and objective tests. The objective measurements were performed on internal frankfurter color only, while sensory panelists were asked to evaluate both skin and internal color together. Skin color may have altered the panelists' overall evaluation of frankfurter color. The objective test measured lightness and redness, while the sensory panel was asked to evaluate the samples for the typical pink chicken

Fig. 1—Surface aerobic plate counts of chicken frankfurters during storage at 4°C.
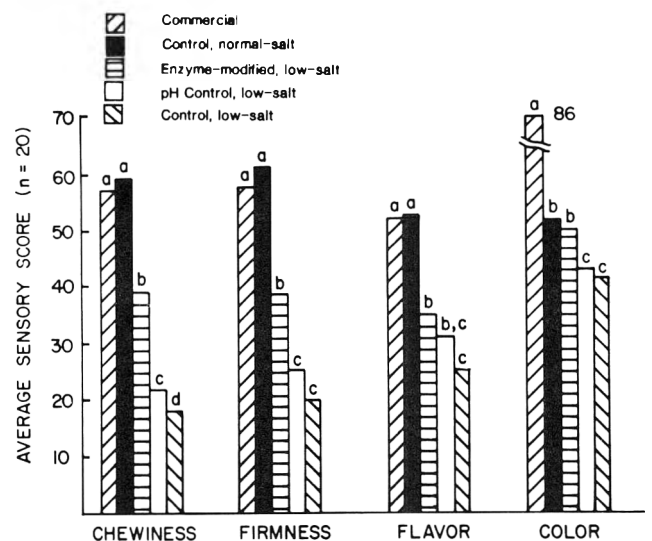
Fig. 2—Average sensory panel scores for texture (chewiness and firmness), flavor and color of chicken frankfurters (Score = 50 indicates samples were rated same as reference).

Table 6—Internal L (lightness) and a_L (redness) values of nonreheated and reheated chicken frankfurters[a]

Treatment	Nonreheated[2]		Reheated	
	L	a_L	L	a_L
Control, normal-salt	62.4[e]	7.7[e]	61.2[e]	7.6[e]
Control, low-salt	62.1[e]	8.5[b]	60.1[e]	8.6[b]
pH Control, low-salt	63.0[e]	6.8[c]	61.7[e]	7.2[e]
Enzyme-modified, low-salt	62.8[e]	7.1[c]	61.7[e]	7.0[e]
Commercial	51.8[b]	11.6[d]	51.8[c]	12.1[c]

[a] Values are the average of two determinations on each of three frankfurters from duplicate batches.
[b-e] Means in the same column followed by the same letter did not differ significantly (P < 0.05).

Table 7—Texture values for nonreheated chicken frankfurters[a]

Treatment	Rupture Force[2] (N)	Compression Slope (N/sec)	Skin Strength (N)
Control, normal-salt	19.6[d]	3.14[d]	7.26[d]
Control, low-salt	7.8[b]	1.47[b]	3.73[b]
pH Control, low-salt	10.9[b]	1.77[b]	3.14[b]
Enzyme-modified, low-salt	16.0[c]	2.35[c]	5.89[b,d]
Commercial	19.6[d]	3.14[d]	7.85[d]

[a] Values are the average of seven determinations on each of three frankfurters from duplicate batches.
[b,c,d] Means in the same column followed by the same letter did not differ significantly (P < 0.05).

frankfurter color. However, the difference in color between the controls and enzyme-modified frankfurter was slight. Any detrimental effect on frankfurter color caused by the manufacturing procedures could be masked by the use of paprika and its oleoresin.

Frankfurter texture

Voisey et al. (1975) reported that firmness and chewiness were the most important parameters for evaluating frankfurter texture by a sensory panel and found that instrumental measurements of compression rupture force and compression slope were highly correlated with sensory chewiness (r < 0.89) and firmness (r < 0.89), respectively.

In both the objective (Table 7) and sensory tests (Fig. 2), the enzyme-modified low-salt frankfurters showed significantly greater firmness and chewiness values than the low-salt and pH controls but were significantly lower in these parameters than the normal salt control and commercial frankfurters. The low-salt and pH control frankfurters had a coarse grainy textural appearance, while the other frankfurters were fine grained.

The skin strength of the low-salt and pH control was significantly lower than the normal-salt and commercial frankfurters when measured with the Fudoh Rheometer (Table 7). The skin strength of the enzyme-modified low-salt frankfurter was intermediate and did not differ significantly from either group. The skin of the low-salt and pH controls was very soft, and it was very difficult to remove the casings without tearing the skin.

Salt is added during the production of a processed meat product to solubilize the myofibrillar proteins so they will exhibit their functional responses, which include water and fat binding and the formation of a desirable texture (Acton et al., 1983). The results of the textural evaluation indicate that enzymatic modification can partially substitute for the role of salt by solubilizing the myofibrillar proteins, allowing them to produce the desired functional response in a processed meat product. However, if frankfurters made from MDF are to gain consumer acceptance, it is important that texture of frankfurters made with enzyme-modified fowl approach that of the normal-salt control. Therefore, the effect of greater salt concentrations in combination with enzyme treatment should be investigated. It may be possible to improve the texture of the enzyme-modified low-salt MDF frankfurters with only a small increase in the salt content.

Frankfurter flavor

All low-salt frankfurter treatments were rated as having significantly less chicken frankfurter flavor than the commercial and normal-salt control frankfurters (Fig. 2). Consumers find frankfurter flavor less desirable when frankfurters are produced with less salt (Olson and Terrell, 1981). If a low-salt frankfurter is to gain consumer acceptance, it will be necessary to develop spice formulations to substitute for the salty flavor.

Proteolysis has been reported to produce a bitter off-flavor in some protein hydrolyzates (Ney, 1979). Neither pH adjustment nor enzymatic modification affected frankfurer flavor adversely, as these samples were not rated below the low-salt control.

The results of this study indicate that enzymatic modification of meat proteins can be used from a functional standpoint to replace a portion of the salt used in frankfurter production. Further work is necessary, however, to evaluate potential microbial problems and to overcome indicated sensory shortcomings prior to industry acceptability.

REFERENCES

Acton, J.C., Ziegler, G.R., and Burge, D.L. 1983. Functionality of muscle constituents in the processing of comminuted meat products. Crit. Rev. Food Sci. Nutr. 18: 99.
Anon. 1983. Changing diet dictates new meat products. Meat Processing, August, p. 47.
AOAC. 1980. "Official Methods of Analysis," 13th ed. Association of Official Analytical Chemists, Washington, DC.
Ayres, J.C., Mundt, J.O., and Sandine, W.E. 1980. "Microbiology of Foods," (Ed.) B.S. Schweigert. W.H. Freeman and Co., San Francisco, CA.
Clark, W.S., Brazis, A.R., Fowler, J.L., Johns, C.K., and Nelson, F.E. 1978. Standard plate count method. In "Standard Methods for the Examination of Dairy Products." (Ed.) E.H. Martin, p. 77. Am. Public Health Assoc., Washington, DC.
Cook, C.F. 1980. Turkey: The natural facts. Louis Rich, Inc., West Liberty, IA.
Forrest, J.C., Aberle, E.D., Hedrick, H,B., Judge, M.D., and Merkel, R.A. 1975. "Principles of Meat Science." W.H. Freeman and Co., San Francisco, CA.
Hand, L.W., Terrell, R.N., and Smith, G.C. 1982. Effects of chloride salts on physical, chemical and sensory properties of frankfurters. J. Food Sci. 47: 1800.
IFT. 1980. Dietary salt. A scientific summary by the Institute of Food Technologists expert panel on food safety and nutrition and the committee on public information. Food Technol. 34: 85.
Kotula, A.W., Ayres, J.C., Huhtanen, C.N., Stern, N.J., Stringer, W.C., and Tompkin, R.B. 1980. Guidelines for microbiological evaluation of meat. Proc. Reciprocal Meat Conference, Am. Meat Sci. Assoc., Chicago, IL, p. 65.
Kramlich, W.E. 1971. Sausage products. In "The Science of Meat and Meat Products," (Ed.) J.F. Price and B.S. Schweigert, p. 485. W.H. Freeman and Co.; San Francisco, CA.
Lawrie, R.A. 1979. "Meat Science," 3rd ed. Pergamon Press, New York, NY.
Lee, Y.B., Hargus, G.L., Kirkpatrick, J.A., Berner, D.L., and Forsythe, R.H. 1975. Mechanism of lipid oxidation in mechanically deboned chicken meat. J. Food Sci. 40: 964.
Ney, K.H. 1979. Bitterness of peptides, amino acid composition and chain length. In "Food Taste Chemistry," (Ed.) J.C. Boudreau, p. 150. Am. Chem. Soc., Washington, DC.
Olson, D.G. 1982. Salt for processing can be cut by only one quarter. Natl. Provisioner, April 17, 18: 67.
Olson, D.G. and Terrell, R.N. 1981. Sensory properties of processed meats using various sodium salt substitutes. Proc. Meat Ind. Res. Conf., p. 59. Am. Meat Inst., Arlington, VA.
Puolanne, E.J. and Terrell, R.N. 1983a. Effects of salt levels in prerigor blends and cooked sausages on water binding, released fat and pH. J. Food Sci. 48: 1022.
Puolanne, E.J. and Terrell, R.N. 1983b. Effects of rigor-state, levels of salt and sodium tripolyphosphate on physical, chemical and sensory properties of frankfurter-type sausages. J. Food Sci. 48: 1036.
Richardson, T. 1977. Functionality changes in proteins and protein derivatives in foods. In "Food Proteins. Improvement Through Chemical and Enzymatic Modification," (Ed.) R.E. Feeney and J.R. Whitaker, p. 67. Am. Chem. Soc., Washington, DC.

Effects of Internal Temperature and Thickness on Palatability of Pork Loin Chops

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─────────ABSTRACT─────────

Three thicknesses (2.54, 1.90 and 1.27 cm) of pork chops were cut alternately from 20 paired loins of similar quality. Chops were cooked on Farberware grills or in a convection oven to internal temperatures of 60, 70 and 80°C for sensory and objective evaluations. Juiciness, percent moisture and tenderness decreased and cooking losses increased ($P < 0.05$) as temperature increased for both cooking methods. Pork flavor intensity increased in oven-prepared chops, while no change was observed in grilled chops at each level of increased temperature. Thickness had no effect ($P > 0.05$) on tenderness, juiciness or total cooking loss of oven-prepared or grilled chops. The thickest chops received the highest ($P < 0.05$) pork flavor scores.

INTRODUCTION

A STUDY of the consumer acceptability of pork chops by Hendrix et al. (1963) showed that 57% of the chops were less than totally acceptable, with "not tender enough" and "not juicy enough" being the most common criticisms. Consumer complaints have initiated many studies dealing with the influence of cooking procedures on meat palatability. Webb et al. (1961), Weir et al. (1963), Carlin et al. (1965) and Pengilly and Harrison (1966) observed increased juiciness and cooked yields of pork loin roasts with decreased internal temperatures. Bramblett et al. (1970) reported cooking time and yields of boneless fresh hams and shoulders to be favorably affected by decreasing doneness temperatures. The current recommended doneness temperature for pork is 75°C (American Meat Science Association and National Live Stock and Meat Board, 1978) which corresponds to well done beef and is approximately 20°C above the 55°C thermal death of *Trichinella spiralis* (Ranson and Schwartz, 1919; Otto and Abrams, 1939).

Emerson et al. (1964) found that consumer preference is greatly influenced by size of cut, as consumers selected chops from carcasses with the largest loin eye area first. Consumer preference of chop thickness was evaluated by Clarke et al. (1983) who concluded that consumers preferred chops between 1.3 and 2.5 cm when shown pictures of chops attached to plastic foam slices of varying thicknesses.

The present study was designed to evaluate the effects of chop thickness and internal temperature on the palatability of pork loin chops prepared in a convection oven or broiled.

MATERIALS & METHODS

TEN PAIRED LOINS were utilized for each cooking method and were selected from pork carcasses of similar quality at the University of Illinois Meat Science Laboratory. A 38 cm portion of the longissimus muscle was removed immediately posterior to the spinalis dorsi and scored for color, firmness and marbling on both cut surfaces using the Wisconsin Pork Quality Standards (1963). Loins were frozen (−40°C) in plastic lined freezer paper for 48 hr and

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then cut, while frozen, into alternating 2.54, 1.90 and 1.27 cm thick chops (18 chops per loin). Chops of similar thicknesses were randomly assigned across temperature treatments (60, 70 and 80°C) in such a manner that all animals were represented once per temperature by thickness treatment resulting in a factorial arrangement of treatments. Chops were trimmed to 0.6 cm of external fat, individually wrapped and returned to the −40°C freezer. Raw muscle samples for pH determination, percent bound water and proximate analysis (grill method only) were removed from the loin and held in a −40°C freezer for subsequent evaluation.

Chops for sensory evaluation were thawed (24 hr at 6°C), weighed and cooked to the appropriate doneness temperature using copper constantan thermocouple wires with a Campbell CR5 digital temperature recorder. Chops were prepared over preheated Farberware Open Hearth grills or over pans in a preheated 177°C South Bend convection oven. The chops were placed on the grill or in the oven at different times to ensure that all chops reached the desired internal temperature at approximately the same time. After reaching the designated temperature, the chops were removed, weighed and wrapped in aluminum foil until served (approximately 5 min) to a six member taste panel for evaluation of sensory characteristics. The taste panel was composed of faculty and graduate students who have previous sensory evaluation experience. Tenderness, juiciness, pork flavor intensity and off flavor intensity were scored on an unstructured scale ranging from 0 to 15, where 0 = extremely tough, dry, bland or extreme off flavor, and 15 = extremely tender, juicy, flavorful or no off flavor. All samples from one animal were cooked per panel session to help minimize day to day variation in preparation of the chops. Transparent, red plastic panels were placed over the lighting fixtures in the sensory evaluation room to minimize panel perception of cooked color differences.

Cooking loss

Total cooking loss for grilled and oven-prepared chops was calculated as the percentage change in chop weight after cooking.

Objective evaluations

Chops used for determination of Warner-Bratzler shear value and proximate analysis were thawed 24 hr at 6°C, cooked to the desired internal doneness temperature and allowed to cool to room temperature. Four 1.27 cm cores were removed (parallel to the muscle fibers) and sheared twice for a total of eight shear values per chop to obtain the reported mean shear value. The remaining portion of each chop was ground twice through a 5 mm plate for moisture determinations via the method described by Koniecko (1979). Fat was extracted from the moisture free samples with repetitive washes of warm chloroform-methanol (2:1) over a 24 hr period (AOAC, 1980). The weight loss of the solvent free samples was recorded as the total extractable fat.

Raw muscle characteristics

Raw muscle samples were thawed overnight and pH (Koniecko, 1979) and percent bound water (Wierbicki and Deatherage, 1958) determined. Proximate analysis (grill method only) was performed as previously described.

Statistical analysis

The results of the sensory evaluation and the physical and chemical determinations were analyzed using the statistical analysis system (SAS, 1982) for analysis of variance and Duncan mean separation analysis.

RESULTS & DISCUSSION

MEANS AND STANDARD DEVIATIONS of raw muscle characteristics are presented in Table 1. Mean marbling and color scores were close to the ideal value of 3; however, loins were slightly less firm than ideal (Wisconsin Pork Quality Standards, 1963). Cut surfaces of the longissimus muscle were scored after removal from the intact loin, which may account for lower firmness scores. Mean pH and percent bound water values were typical of normal pork muscle.

Means and standard errors for cooking and palatability traits of chops oven-prepared or grilled to 60, 70 and 80°C are presented in Table 2. As expected, total cooking losses increased and percent moisture decreased ($P < 0.05$) with increased doneness temperatures for both cooking methods. These results were in agreement with the findings of Weir et al. (1963), Carlin et al. (1965), Webb et al. (1961) and Pengilly and Harrison (1966). Percent lipid on a dry basis was greater ($P < 0.05$) for chops grilled to 80° versus 60° and 70°C, indicating an inverse relationship between percent lipid and percent moisture. Percent lipid did not differ ($P > 0.05$) with increased internal temperature in the oven-prepared chops; however, an increase in percent lipid was observed as internal temperature increased.

Mean values for sensory panel tenderness scores for oven-prepared chops were lower ($P < 0.05$) with increased internal temperature. Webb et al. (1961) obtained similar results, while Weir et al. (1963), Carlin et al. (1965) and Pengilly and Harrison (1966) found that tenderness in loin roasts did not vary significantly with changes in inter-

nal doneness temperature. A significant decrease in tenderness of chops grilled to 80°C as compared to 60° or 70°C ($P < 0.05$) was found by sensory panel evaluation. The decreased tenderness was influenced by the only thickness temperature interaction observed while no significant three-way interactions were found in the study. The 2.54 cm chops increased in tenderness from 60° to 70°C, while the thinner chops decreased in tenderness with increased temperature. Juiciness values as determined by the sensory panel decreased ($P < 0.05$) with increased internal temperature for both oven-prepared and grilled chops. These results were consistent with those of Weir et al. (1963), Carlin et al. (1965), Webb et al. (1961) and Pengilly and Harrison (1966).

Pork flavor was more intense ($P < 0.05$) with increased internal temperature for the oven-prepared chops. Webb et al. (1961) attributed higher flavor scores to the preconditioning of panel members to prefer the pork flavors developed at higher temperatures because pork has traditionally been prepared to high temperatures. The oven-prepared chops cooked to higher internal temperatures had more surface browning which may have contributed to the higher pork flavor values. Cooking time may have also affected pork flavor development since those chops cooked to higher temperatures remained in the oven longer. Pork flavor intensity of grilled chops was not affected by increased temperature. A more uniform surface browning was observed at all three temperature endpoints in the grilled chops versus the oven-prepared chops. Chops oven-prepared and grilled to an internal temperature of 60°C were perceived as having greater ($P < 0.05$) off flavor intensity than chops prepared to 70° and 80°C.

Warner-Bratzler shear values of grilled loin chops were not ($P > 0.05$) affected with increased internal temperature; however, a difference ($P < 0.05$) was observed in the oven-prepared chops with those cooked to 80°C possessing the highest shear values. This result corresponds with the sensory panel findings where oven-prepared chops cooked to 80°C were the least tender.

Means and standard error values for various traits of oven-prepared and grilled loin chops of three thicknesses are presented in Table 3. Total cooking losses were not different ($P > 0.05$) for oven-prepared or grilled loin chops; however, a decrease ($P < 0.05$) in percent moisture was observed in the 1.27 cm chops compared to the thicker chops for both cooking methods. No differences ($P > 0.05$) in percent lipid (dry matter basis) were observed in oven-prepared chops of three thicknesses. On the other hand, the grilled 1.90 cm chops had ($P < 0.05$) less percent lipid than the 1.27 cm chops.

Table 1—Mean and standard deviation values for raw muscle characteristics of chops for oven and grill preparation

| Trait | Kind of preparation | | | |
| | Oven | | Grill | |
	Mean	S.D.	Mean	S.D.
pH	5.38 ± 0.16		5.48 ± 0.15	
Marbling[a]	2.90 ± 0.49		2.80 ± 0.60	
Color[a]	2.90 ± 0.30		2.90 ± 0.54	
Firmness[a]	2.55 ± 0.35		2.50 ± 0.50	
Bound water (%)[b]	62.46 ± 4.31		66.34 ± 6.00	

[a] Based on the Wisconsin Pork Quality Standards (1963). Color: 1 = pale, grayish-white; 3 = graylsh-pink; 5 = dark red. Marbling: 1 = practically devoid; 3 = modest; 5 = abundant. Firmness: 1 = extremely soft and watery; 3 = moderately firm and dry; 5 = very firm and very dry.
[b] Determined according to the formula of Wierbicki and Deatherage (1958).

Table 2—Means and standard error values for various traits of pork loin chops oven-prepared or grilled to three internal temperatures.

	Kind of preparation							
	Oven				Grill			
	Internal temperature (°C)				Internal temperaure (°C)			
Trait	60	70	80	S.E.	60	70	80	S.E.
Total cooking loss (%)[d]	21.62[a]	29.32[b]	36.68[c]	±0.61	26.16[a]	32.97[b]	41.66[c]	±0.91
Moisture (%)	66.73[a]	63.96[b]	60.27[c]	±0.26	67.44[a]	64.62[b]	60.24[c]	±0.58
Lipid (%)[e]	15.76[a]	17.06[a]	17.47[a]	±0.50	11.67[a]	12.30[a]	14.29[b]	±0.24
Tenderness[f]	10.90[a]	10.15[b]	8.49[c]	±0.18	11.26[a]	10.82[a]	9.14[b]	±0.31
Juiciness[f]	11.77[a]	9.69[b]	6.96[c]	±0.24	11.37[a]	9.42[b]	6.06[c]	±0.28
Pork flavor intensity[f]	8.51[a]	9.93[b]	11.40[c]	±0.21	9.79[a]	10.12[a]	10.17[a]	±0.19
Off flavor intensity[g]	11.76[a]	12.33[ab]	13.10[b]	±0.19	13.32[a]	13.74[b]	13.96[b]	±0.13
Warner-Bratzler shear value[h]	3.47[a]	3.49[a]	4.34[b]	±0.37	3.26[a]	2.99[a]	3.15[a]	±0.12

[abc] Mean values in the same row bearing unlike superscripts differ significantly ($P < 0.05$).
[d] Cooking losses calculated as a percentage of raw chop weight.
[e] Dry matter basis.
[f] Means derived from sensory panel scores with possible range from 0 – 15 where 0 = extremely tough, dry or bland, and 15 = extremely tender, juicy or flavorful.
[g] Means derived from sensory panel scores with possible range from 0 – 15, where 0 = extreme off flavor and 15 = no off flavor.
[h] kg per 1.27 cm core.

Trait	Kind of preparation							
	Oven				Grill			
	Thickness (cm)				Thickness (cm)			
	1.27	1.90	2.54	S.E.	1.27	1.90	2.54	S.E.
Total cooking loss (%)[d]	29.82[a]	29.76[a]	28.04[a]	±0.61	33.18[a]	33.40[a]	34.23[a]	±0.91
Moisture (%)	62.14[a]	64.14[b]	64.67[b]	±0.26	62.73[a]	65.32[b]	64.25[b]	±0.58
Lipid (%)[e]	17.24[a]	16.98[a]	16.07[a]	±0.50	13.28[a]	12.49[b]	12.73[ab]	±0.24
Tenderness[f]	9.96[a]	9.77[a]	9.81[a]	±0.18	10.51[a]	10.44[a]	10.27[a]	±0.31
Juiciness[f]	9.20[a]	9.34[a]	9.87[a]	±0.24	8.65[a]	9.07[a]	9.13[a]	±0.28
Pork flavor intensity[f]	9.68[a]	9.73[ab]	10.44[b]	±0.21	9.59[a]	10.07[b]	10.42[b]	±0.19
Off flavor intensity[g]	12.31[a]	12.06[a]	12.82[a]	±0.19	13.31[a]	13.82[b]	13.88[b]	±0.13
Warner-Bratzler shear value[h]	3.82[a]	3.77[a]	3.69[a]	±0.37	3.34[a]	3.02[b]	3.04[b]	±0.12

[abc] Mean values in the same row bearing unlike superscripts differ significantly ($P < 0.05$).
[d] Cooking losses calculated as a percentage of raw chop weight.
[e] Dry matter basis.
[f] Means derived from sensory panel scores with possible range from 0 – 15, where 0 = extremely tough, dry or bland, and 15 = extremely tender, juicy or flavorful.
[g] Means derived from sensory panel scores with possible range from 0 – 15, where 0 = extreme off flavor and 15 = no off flavor.
[h] kg per 1.27 cm core.

Tenderness and juiciness sensory panel values did not differ ($P > 0.05$) with different thicknesses of oven-prepared or grilled chops. Pork flavor intensity was ($P < 0.05$) greater for the 2.54 cm grilled chops. The greater flavor intensity values observed for the thicker chops may have been enhanced by greater surface browning since the thicker chops were cooked longer to reach the same internal temperature.

Off flavor intensity and Warner-Bratzler shear values did not differ ($P > 0.05$) for oven-prepared chops of different thicknesses. In the grilled chops, significant ($P < 0.05$) off flavor intensity and higher shear values were observed in 1.27 cm loin chops.

CONCLUSIONS

IT APPEARS from this study that reduced internal doneness temperatures have favorable effects on juiciness, tenderness and cooked yield of chops. Benefits of the 60°C internal temperature may be best realized on thicker cut chops, or by grilling, as pork flavor intensity is maintained. The surface of thin chops appeared undercooked when oven-prepared to 60°C, and overcooked when grilled to 80°C. The palatability of pork loin chops is improved when (1) internal temperatures of thick (>1.90 cm) chops is reduced to less than 70°C, (2) internal temperature of thin (<1.90 cm) chops is reduced to 70°C, and (3) chop thickness is greater than 1.27 cm.

REFERENCES

AOAC. 1980. "Official Methods of Analysis," 13th ed. Association of Official Analytical Chemists, Washington, DC.

American Meat Science Association and National Live Stock and Meat Board. 1978. Guidelines for cookery and sensory evaluation of meat. Am. Meat Sci. Assoc., Chicago, IL.
Bramblett, V.D., Judge, M.D., and Harrington, R.B. 1970. Effect of temperature and cut on quality of pork roast. J. Am. Diet. Assoc. 57: 132.
Carlin, A.F., Bloemer, D.M., and Hotchkiss, D.K. 1965. Relation of oven temperature and final internal temperature to quality of pork loin roasts. J. Home Econ. 57: 442.
Clarke, A.D., Ramsey, C.B., Hornsby, V.E., and Davis, G.W. 1983. Consumer preferences for fat and chop thickness in fresh pork chops. Proc. Recip. Meat Conf. 36: 193.
Emerson, J.A., Pearson, A.M., Hoefer, J.A., Magee, W.T., and Bratzler, L.J. 1964. Effect of slaughter weight upon the processing characteristics, quality and consumer acceptability of pork carcasses and cuts. J. Anim. Sci. 23: 436.
Hendrix, J., Baldwin, R., Rhodes, V.J., Stringer, W.C., and Naumann, H.D. 1963. Consumer acceptance of pork chops. Res. Bull. No. 834, Univ. of Missouri Agr. Exp. Sta., Columbia, MO.
Koniecko, E. 1979. "Handbook for Meat Chemists." Avery Publishing Group, Inc., Wayne, NJ.
Otto, G.F. and Abrams, E. 1939. Quantitative studies on the effect of heat on trichinae (Trichinella spiralis) larvae. Am. J. Hygiene 29: 115.
Pengilly, C.I. and Harrison, D.L. 1966. Effect of heat treatment on the acceptability of pork. Food Technol. 20: 98.
Ranson, B.H. and Schwartz, B. 1919. Effects of heat on trichinae. J. Agr. Res. 17: 201.
SAS Institute, Inc. "SAS User's Guide: Statistics," 1982 ed. SAS Institute, Inc., Cary, NC.
Webb, N.L., Webb, N.B., Cedarquist, D., and Bratzler, L.J. 1961. The effect of internal temperature and time of cooking on the palatability of pork loin roasts. Food Technol. 15: 371.
Weir, C.E., Pohl, C., Auerbach, E., and Wilson, G.D. 1963. The effect of cooking conditions upon the yield and palatability of pork loin roasts. Food Technol. 17: 95.
Wierbicki, E. and Deatherage, F.E. 1958. Determination of water-holding capacity of fresh meats. J. Agr. Food Chem. 6: 387.
Wisconsin Pork Quality Standards. 1963. Special Bull. No. 9, Exp. Sta. and Ext. Service, Univ. of Wisconsin, Madison, WI.

Fate of *Staphylococcus aureus* in Reduced Sodium Fermented Sausage

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―――――――――――― ABSTRACT ――――――――――――

Sausage was prepared using three levels of sodium chloride. These sausages were made with a commerical starter culture, and with an inoculum of *Staphylococcus aureus,* strain Z-88. The sausages containing lower amounts of salt showed faster fermentations at two temperatures, 24°C and 38°C. At both temperatures the sausages containing 1.65% salt showed the least amount of staphylococcal growth followed by the sausages containing 2.475% and 3.3% salt respectively.

INTRODUCTION

FERMENTATION as a means of preservation of meat is one of the oldest forms of preservation. Salt plays an important role in the stability of the finished fermented sausage product, which depends partly on a moisture level of water activity at which spoilage or pathogenic organisms will not grow. Salt aids in this preserving effect by binding free water and decreasing the water available for unwanted growth. In dry sausage, salt is added primarily as a flavoring agent, but it is also considered to inhibit undesirable bacteria during the early stages of fermentation while allowing the salt-tolerant lactic acid bacteria to grow and produce lactic acid (Pederson, 1979; Sebranek, 1982).

Staphylococcus aureus can multiply and produce toxin during the initial stage of fermentation, given the proper conditions. *S. aureus* has been responsible for food poisoning incidents in many types of foods including fermented sausage. The first effect that lactic acid bacteria have on *S. aureus* is the production of lactic acid which inhibits *S. aureus.* However, fermentation by the bacteria present naturally or added to the meat controls *S. aureus* in a way separate from simply lowering the pH of the product. The lactic acid bacteria, when present in large numbers, have been shown in numerous experiments to be able to repress *S. aureus* (Peterson et al., 1962; Haines and Harmon, 1973; Tatini et al., 1971; Raccach and Baker, 1978).

S. aureus is one of the more tolerant organisms to high concentrations of salt and reduced water activity (a_w). Up to 10% NaCl does not essentially alter the ratio of enterotoxin A formation to growth of *S. aureus,* although the quantity of enterotoxin decreases as the salt concentration increases (Markus and Silverman, 1970). This decrease in enterotoxin produced is apparently due to the increase in generation time for the organism with decreasing water activity in the medium. Troller (1972) reported an increase in generation time from 37 min to 150 min with a shift in a_w from 0.98 to 0.89 in a medium whose a_w was adjusted with NaCl. Production of enterotoxin B is more repressed by salt concentration than is enterotoxin A production, because salt slows the growth rate of *S. aureus* and enterotoxin B is produced as the culture reaches the stationary growth phase (Pereira et al., 1982; Troller, 1972).

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In recent years, there has been much research done which indicates that a high level of dietary sodium intake might have very serious health risks for approximately 20% of the American population, especially in terms of high blood pressure and related disease states. These findings, in combination with increased marketing and advertising for the benefits of eating foods lower in sodium or salt content, have brought about increased demand for processed meat products containing less salt.

Although dry fermented sausage does not represent a large portion of the modern American diet, it does represent a type of meat product which is very high in salt content. Therefore, the purpose of this project was to determine if salt reduction in a fermented sausage product has any effect on *Staphylococcus aureus* or a starter culture of lactic acid bacteria and if so, which organism is affected most and possible implications regarding safety of the product.

MATERIALS & METHODS

Experimental design

Two fermentation temperatures were used, a high temperature (38°C) and a low temperature (24°C). Three trials were done for each, but only two replications of each were analyzed statistically because of weak activity of the starter culture in the first replication.

Experimental procedure

All of the meat used in this study was obtained from the Iowa State University Meat Laboratory and processed at the same time. Lean pork and pork trim were ground through a 4.76 mm (3/16 in) plate and mixed to give 25% fat. The meat was packaged in polyethylene freezer bags and kept frozen in a blast freezer (−20°C) until use. Meat was taken from the freezer 4 days prior to sausage manufacture and placed in a cooler at 5°C for 3 days and then the bags of meat were placed in pans of cold water for 1 day in the 5°C cooler before sausages were made. This method was effective in providing meat ready for mixing.

The dry ingredients used in this work were weighed into individual bags prior to making sausage and are listed in Table 1. All batches received 1.1 mL of Lactacel 75 starter culture (Microlife Technics, Sarasota, FL). The batches to be inoculated with *S. aureus* received 0.5 mL of a 24-hr culture of Z-88 in Brain Heart Infusion broth (Difco Laboratories, Detroit, MI). Cultures were previously dispensed into 99 mL of sterile 0.1% peptone water for dispersion.

Sausage was made in batches starting with the control batch which contained 3.3% NaCl and starter culture, but no *S. aureus.* The batches containing *S. aureus* were made in order of increasing salt levels to decrease the percentage of carryover from the previous batch. All batches were made in a mixer (Model H-600T, Hobart Manufacturing Co., Troy, OH). Salt concentration in the sausage samples was determined with a Fisher Accumet Selective Ion Analyzer (Model 750) following the method developed by Orion Research Inc. (1980).

After all batches were mixed, the meat from the control batch was placed in a Vogt manual sausage stuffer. The first kilogram was discarded and the remainder stuffed into 65 mm (diam) fibrous casings (Teepak, Chicago, IL). Each sausage was approximately 27 cm in length and weighed approximately 0.35 kg. Twelve sausages were stuffed from each batch and the remainder of the batch discarded. Next, the batches containing *S. aureus* were stuffed in order of increasing salt level. The first kilogram was extruded and dis-

Table 1—Dry ingredients used in sausage

Ingredient	Amount	Per cent of meat wt
Dextrose	27.20g	0.500%
Ground black pepper	13.60g	0.250%
Whole black pepper	6.80g	0.120%
Garlic powder	1.60g	0.030%
Sodium nitrite	0.85g	0.016%

carded to "flush" the stuffer of remaining material from the previous batch.

The sausages were hung in a controlled temperature, controlled humidity, Modu-Lab Room 914A-HD (Lab-Line Instruments, Inc., Chicago, IL). All fermentations were at 90-95% relative humidity. Low temperature fermentation was at 24°C (75°F) and high temperature fermentation was at 38°C (100°F).

During low temperature fermentation, 4 Orion probe electrodes (Model 9163, Orion research Inc., Cambridge, MA) were standardized against pH 4.0 and 7.0 buffers, and inserted into a sausage from each batch after 12 hr of fermentation. During high temperature fermentation, the probe electrodes were inserted after 2 hr of fermentation. These electrodes were connected to a London six channel automatic switch (Type SAS-1), which monitored each electrode for 5 min and then switched to the next electrode. The pH was registered with a Radiometer (Model PHM28C) pH meter. A recording of the signals to the pH meter was made with a Bausch and Lomb lab recorder (Model V. O. M. 7) running at a chart speed of 0.25 in/5 min. Fermentation was considered complete when all recordings reached a plateau. Upon completion of fermentation, the chamber was set to 18.3°C (65°F) and 80-85% relative humidity. This drying phase was continued for 1 wk.

Microbial analysis

For microbial analyses, a sample was taken from the meat initially, and a representative sample was taken from each batch at the time of sausage manufacture, every 12 hr during fermentation, and daily thereafter throughout the drying period. Each sample consisted of 30g of meat taken aseptically from the outermost 1 cm of meat and added to 270 mL of sterile 0.1% peptone water in a blender jar. Serial dilutions were then made in 0.1% peptone water. *Staphylococcus aureus* was enumerated with Baird-Parker medium (BBL and Difco, 24-48 hr at 37°C); lactic acid organisms were enumerated using Lactobacillus Selective (LBS) medium (BBL, 48 hr at 30°C); and aerobic plate counts were made with Trypticase Soy Agar (TSA, BBL, 72 hr at 30°C). Samples were prepared by the pour-plate method for LBS and TSA media, and by the spread-plate method using 0.1 ml aliquots of inoculum for Baird-Parker medium.

RESULTS & DISCUSSION

Low temperature fermentation

At a fermentation temperature of 24°C (75°F) and the normal salt level of 3.3% NaCl, reduction in pH occurred at 17 hr of fermentation (Fig. 1). A drop in pH was recorded at 15 hr for sausage containing 2.475% NaCl and at 13 hr for sausage containing 1.65% NaCl. By reducing the salt concentration by 50%, from 3.3 to 1.65%, a pH decrease was observed in sausages with the lowest salt concentration 4 hr earlier than in sausages with 3.3% salt.

With regard to total fermentation time, the sausages containing 1.65% salt had finished fermenting after an average of approximately 23 hr. Sausages containing 2.475% NaCl were finished fermenting in approximately 28 hr. The two batches made with 3.3% NaCl finished in approximately 33 hr. A reduction in 10 hr in the total fermentation time resulted from reducing the salt concentration by 50%.

The slopes of the straight line portion of the curves for pH decrease were compared statistically, resulting in an F value of 5.945 ($P < 0.05$), confirming that the slopes were not equal. Fig. 1 shows that for 1.65% salt between 14 and 19 hr, the change was more rapid than that for 2.475%
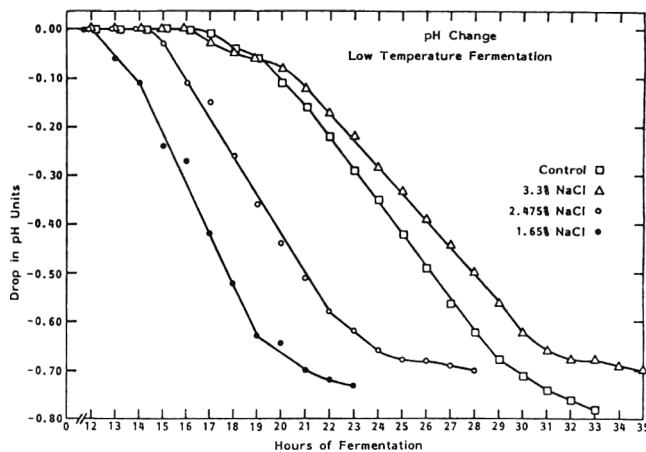
Fig. 1—Change in pH during low temperature fermentation (24°C).
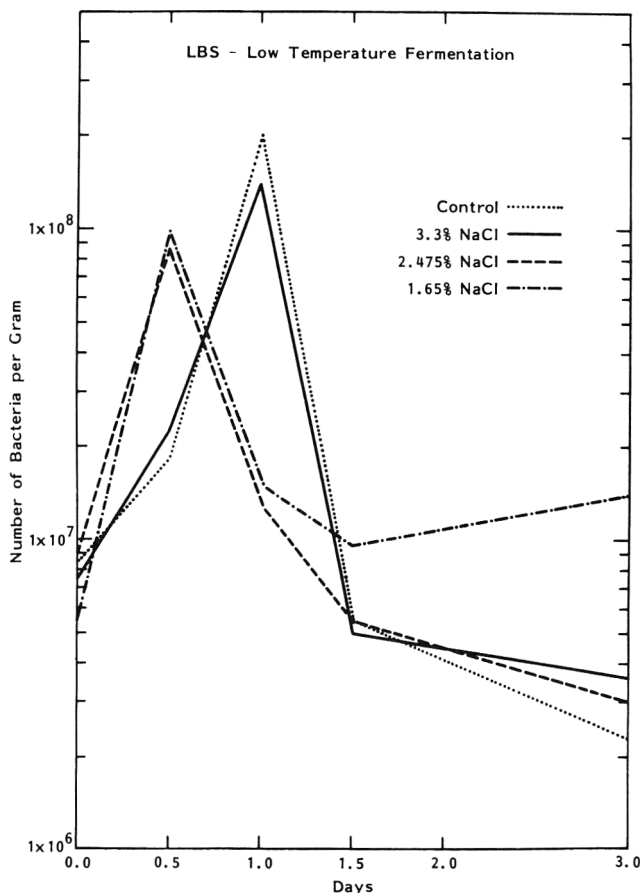
Fig. 2—Numbers of lactic acid bacteria during fermentation at low temperature (24°C).

NaCl at 16-22 hr and for the two batches containing 3.3% salt at 20-30 hr.

Lactic acid bacteria

The plate counts of lactic acid bacteria are presented in Fig. 2 and 3. Fermentation conditions were maintained through 36 hr (1.5 days) and the time shown on the graphs after that represents the drying period. The lower salt concentrations (1.65% and 2.475% salt) allowed the lactic acid bacteria to grow at a faster rate than the two batches containing 3.3% salt. Growth rate was related to the faster drop in pH noted in Fig. 1. Also, at the end of fermentation, the
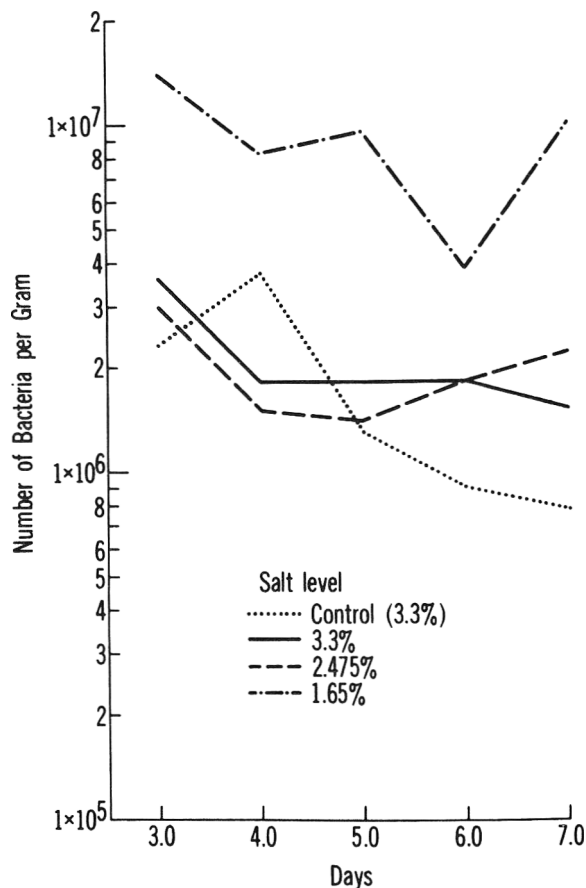
Fig. 3–Numbers of lactic acid bacteria during drying after low temperature (24°C) fermentation.

Fig. 4–Numbers of Staphylococcus aureus during fermentation at low temperature (24°C).

Fig. 5–Numbers of Staphylococcus aureus during drying after low temperature fermentation.

number of lactic acid bacteria surviving after fermentation in sausages made with 1.65% salt was higher than in sausages made with higher salt levels (Fig. 3). This difference was maintained during the short drying phase up to 3 days although all batches showed a generally downward trend in bacterial numbers through this phase. Differences in growth with different salt levels in sausages were significant (P < 0.01) for fermentation and drying.

Staphylococcus aureus

Numbers of staphylococci are shown in Fig. 4 and 5. The staphylococci were generally controlled at 24°C by the relatively low temperature of fermentation, along with rapid growth of the starter culture at this temperature. When a "weak" starter culture was used in preliminary experiments, the *Staphylococcus aureus* overcame the temperature conditions to show moderate growth (Marcy, 1984). The faster growth of the starter culture used in these fermentations prevented *S. aureus* from overcoming the relatively unfavorable temperature and increasing in numbers by even one logarithm. The greatest increase was in sausages made with 3.3% salt, and amounted to an increase of 46%. In a similar product and process, a 20% increase from an initial level of 7.6×10^3 staphylococci/gram was reported (Anon., 1980). Previous work has shown that *S. aureus* does not grow well at low temperature, particularly in a fermented sausage environment, and especially in conjunction with a lactic acid starter culture capable of low temperature fermentation (Scheusner et al., 1973; Anon., 1980). This is the basis for the current "Good Manufacturing Practice" (GMP) designed by the American Meat Institute (1982) which attempts to limit the amount of time that fermented sausage is exposed to temperatures
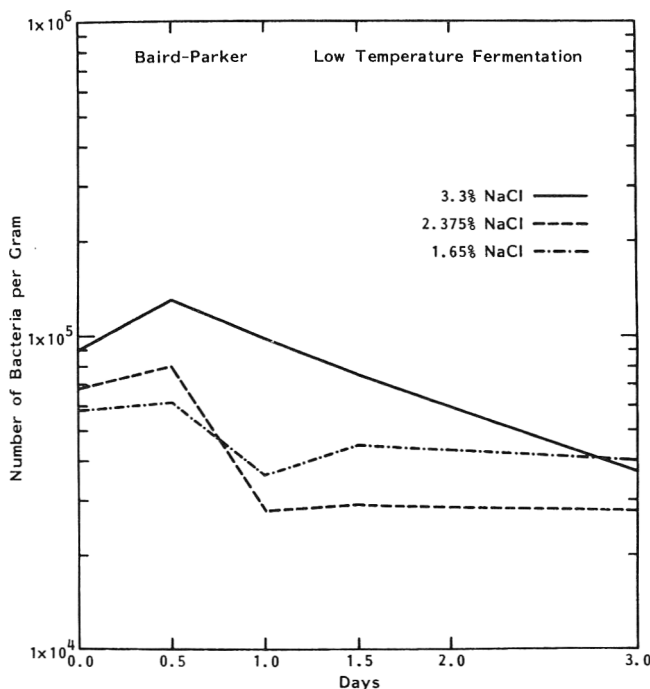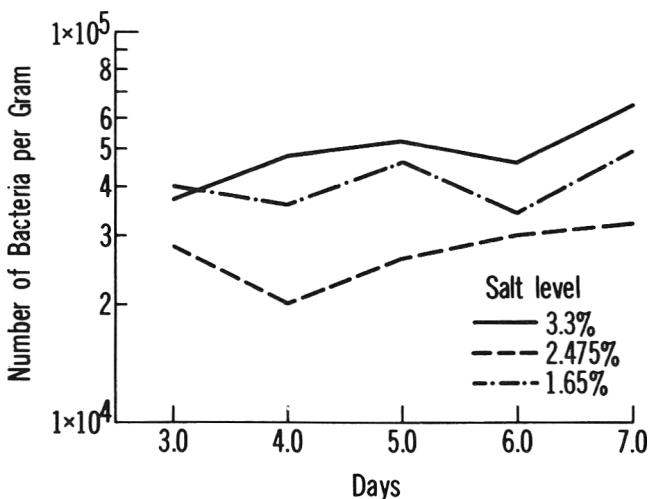
greater than 15.5°C (60°F). *S. aureus* demonstrated a slight upward trend in numbers during drying (Fig. 5) (P < 0.05).

High temperature fermentation

At a fermentation temperature of 38°C (100°F), differences were observed in fermentation rates based on salt concentration (Fig. 6). As in the low temperature fermentation, the sausages containing the lowest salt level showed a response in terms of pH drop first, followed in order by sausages made with 2.475% and 3.3% salt. Reduction in pH was observed in sausages containing 1.65% salt at 5 hr, 2.475% salt at 6 hr, and by 3.3% salt and the control with no inoculated *S. aureus* (3.3% salt) at 7 and 8 hr, respectively.

A comparison of the slopes of the straight line portions of the pH curve gave an F value of 3.168 (P < 0.05) indicat-
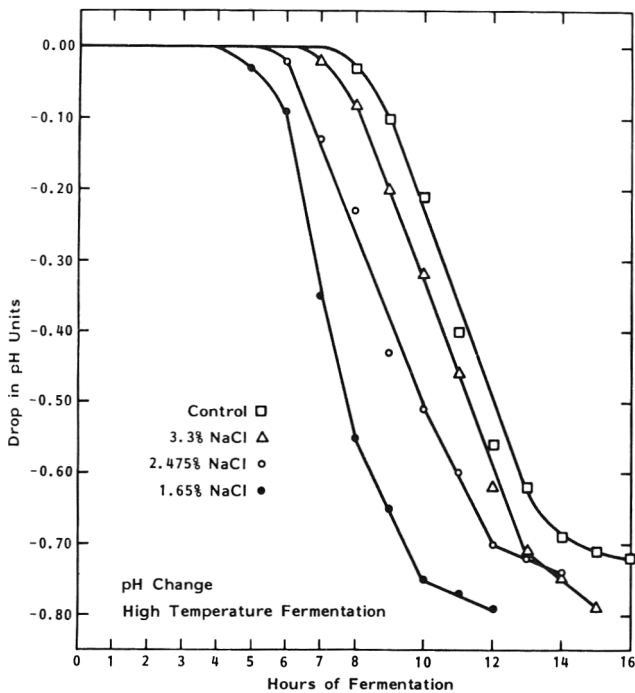
Fig. 6—Change in pH during high temperature fermentation (38°C).

Table 2—Average plate counts of lactic acid bacteria during drying (2-7 days) after high temperature fermentation

	Number of bacteria per gram			
Day	1.65% NaCl	2.475% NaCl	3.3% NaCl	Control
2	7.8×10^6	2.7×10^6	3.9×10^6	2.0×10^6
3	7.0×10^6	4.0×10^6	1.9×10^6	2.4×10^6
4	6.0×10^6	1.8×10^6	3.4×10^6	1.1×10^6
5	1.2×10^7	1.4×10^6	2.2×10^6	7.4×10^5
6	8.4×10^6	1.1×10^6	1.1×10^7	6.6×10^5
7	4.1×10^6	2.0×10^6	5.4×10^6	7.6×10^5

Fig. 7—Numbers of Staphylococcus aureus during fermentation at high temperature (38°C).

Table 3—Average plate counts of Staphylococcus aureus during drying (2-7 days) after high temperature fermentation

	Number of bacteria per gram		
Day	1.65% NaCl	2.475% NaCl	3.3% NaCl
2	1.4×10^5	1.2×10^6	6.6×10^6
3	1.4×10^5	1.2×10^6	5.6×10^6
4	1.2×10^5	8.6×10^5	6.3×10^6
5	1.5×10^5	1.1×10^6	6.0×10^6
6	1.2×10^5	1.1×10^6	1.4×10^7
7	1.3×10^5	1.3×10^6	1.0×10^7

ing again that not all of the slopes were equal. By inspection, the slope for sausages with 1.65% salt (6-10 hr) appeared appreciably steeper than the slopes for 2.475% salt (6-12 hr), 3.3% salt (7-13 hr), and control (8-13 hr).

Lactic acid bacteria

Table 2 presents the average plate counts of lactic acid bacteria during the complete drying phase (2-7 days). The fermentation was so rapid at this temperature that an interval as long as 12 hr for enumeration of lactic acid bacteria during fermentation was inappropriate. The sausages containing 1.65% salt had a higher level of lactic acid bacteria surviving the fermentation than samples containing higher salt concentrations ($P < 0.01$). As in the low temperature fermentation, the lactic acid bacteria showed a general downward trend for all salt levels (Table 2) ($P < 0.05$).

Staphylococcus aureus

Plate counts for S. aureus are presented in Fig. 7 for fermentation (about 0.85 days) and the first two days of drying. Counts after day 3 remained relatively stable through 7 days and the average plate counts for the complete drying phase are given in Table 3. Fig. 7 shows clearly the effect of salt when the temperature is near the optimum for staphylococcal growth. Because of the increase in rate of fermentation, as judged by the slope for pH decline, S.

aureus was allowed to multiply by a factor of only 4 in the sausages containing 1.65% salt while the sausages made with 3.3% salt showed an increase of a factor of 161 ($P < 0.01$). This is in contrast to a report by Microlife Technics (Anon., 1980) which stated an increase in S. aureus of a factor of only 1.3 in sausage containing 3.3% salt and an initial level of 7.4×10^3 S. aureus/gram during an 11 hr fermentation of 35°C.

The effect of salt was linear with respect to the concentration, with an F value of 24.39 ($P = 0.003$). This means that the level of inhibition exerted on S. aureus, either directly or indirectly, was related to the concentration of salt in the product. Reduction in salt increased the inhibition of S. aureus.

A significant point is that at near optimum temperatures for growth of S. aureus, even a 1 hr decrease in the time

required to start producing acid has a very beneficial effect in the control of *S. aureus*. The relationship between the lactic acid bacteria and *S. aureus* is a competitive one. The faster the growth of the lactic acid bacteria and the subsequent production of lactic acid, the greater the degree of inhibition of *S. aureus*.

At the present time, dry, fermented sausage is the only product which has a specified minimum level of salt to be added (3.3%), and this is only in the instance where pork which is noncertified free of trichina is used in the formulation. This, however, may be subject to change. The USDA has proposed that this salt level may be reduced with substitution for control of trichina by specified time-temperature-salt content relationships, which would allow the industry more flexibility in product formulation (USDA, 1983).

SUMMARY

THE WORK PRESENTED here was done to determine effects of reduction in salt in fermented sausage on lactic acid bacteria starter cultures and *Staphylococcus aureus*. Consideration was also given to fermentation control by use of two different temperatures.

Reduction in salt allowed for faster growth and faster production of acid by the lactic acid starter culture at both $24°C$ and $38°C$. Therefore, it is believed that the effect on the lactic acid culture was of a direct nature. The effect of salt reduction on the staphylococci was dependent on the rate of growth and acid production by the lactic acid bacteria. Staphylococci were inhibited in an indirect, but linear relationship to the amount of salt present in the sausage.

Reduction of salt may not be expected to have as much inhibitory effect on high levels of initial staphylococci in a product which is being fermented at a high temperature and without the addition of a commercially prepared lactic acid bacteria starter culture suitable for growth at that temperature. This would probably include processes relying on a "slop-back" inoculum for the starter culture and processes utilizing the lactic acid bacteria naturally occurring in the meat as the source of fermentation. These products should be fermented in accordance with the "Good Manufacturing Practice" of the American Meat Institute, with low temperature as the controlling force on *S. aureus*.

Because of the linear relationship between the salt concentration and the inhibition of *S. aureus* at high temperature, care must be taken to insure that the proper level of salt is added to the product. This is especially true in operations which do not monitor the pH of the sausage during the fermentation.

Our work demonstrated that at decreased salt levels, the pH drop in fermented sausages initiated by lactic starter cultures proceeded at rates that prevented the growth of *S. aureus* at both $24°$ and $38°C$. However, it should be emphasized that the starter culture must be an active fermenting one.

REFERENCES

American Meat Institute. 1982. Good manufacturing practices number two. Fermented dry and semi-dry sausage. American Meat Institute, Arlington, VA.
Anon. 1980. Control of Staphylococcus aureus growth in fermented sausage. Technics/Topics (Microlife Technics, Sarasota, FL) L3(3).
Haines, W.C. and Harmon, L.G. 1973. Effect of selected lactic acid bacteria on growth of Staphylococcus aureus and production of enterotoxin. Appl. Microbiol. 25: 436.
Marcy, J.A. 1984. The effect of reduced salt (NaCl) levels in Genoa salami inoculated with Staphylococcus aureus. M.S. thesis, Iowa State University, Ames, IA.
Markus, Z.H., and Silverman, G.J. 1970. Factors affecting the secretion of staphylococcal enterotoxin A. Appl. Microbiol. 20: 492.
Orion Research, Inc. 1980. Salt in processed meats. Procedure No. 204. Orion Research, Inc. Cambridge, MA.
Pederson, C.S. 1979. Fermented sausage. In "Microbiology of Food Fermentations," 2nd ed., p. 210. AVI Publishing Company, Inc., Westport, CT.
Pereira, J.L., Salzberg, S.P., and Bergdoll, M.S. 1982. Effect of temperature, pH, and sodium chloride concentrations on production of staphylococcal enterotoxins A and B. J. Food Protection 45: 1306.
Peterson, A.C., Black, J.J., and Gunderson, M.F. 1962. Staphylococci in competition. II. Effect of total numbers and proportion of staphylococci in mixed cultures on growth in artificial medium. Appl. Microbiol. 10: 23.
Raccach, M. and Baker, R.C. 1978. Lactic acid bacteria as an antispoilage and safety factor in cooked, mechanically deboned poultry meat. J. Food Protection. 41: 703.
Scheusner, D.L., Hood, L.L., and Harmon, L.G. 1973. Effect of temperature and pH on growth and enterotoxin production by Staphylococcus aureus. J. Milk Food Technol. 36: 249.
Sebranek, J.G. 1982. Using non-meat ingredients for sausage and processed meats. In "Proc. 4th Annual Sausage and Processed Meats Short Course," p. 17. Cooperative Extension Service, Iowa State University, Ames, IA.
Tatini, S.R., Jezeski, J.J., Olson, J.C., Jr., and Casman, E.P. 1971. Factors influencing the production of staphylococcal enterotoxin A in milk. J. Dairy Sci. 54: 312.
Troller, J.A. 1972. Effect of water activity on enterotoxin A production and growth of Staphylococcus aureus. Appl. Microbiol. 24: 440.
USDA. 1983. Trichina control requirements. United States Department of Agriculture. Fed. Register 48: 10065.
Ms received 8/6/84; revised 10/1/84; accepted 10/3/84.

ENZYME MODIFIED LOW-SALT FOWL FRANKFURTERS...

Smith, D.M. and Brekke, C.J. 1984. Functional properties of enzymatically modified beef heart protein. J. Food Sci. 49: 1525.
Smith, D.M. and Brekke, C.J. 1985. Enzymatic modification of the structure and functional properties of mechanically deboned fowl proteins. J. Agric. Food Chem. (in press)
Sofos, J.N. 1983. Effects of reduced salt (NaCl) levels on the stability of frankfurters. J. Food Sci. 48: 1684.
Steel, G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics," McGraw-Hill Book Co., Inc., New York, NY.
Terrell, R.N., Quintanilla, R., Vanderzant, C., and Gardner, F.A. 1983. Effects of reduction or replacement of sodium chloride on growth of Micrococcus, Moraxella and Lactobacillus inoculated ground pork. J. Food Sci. 48: 122.
Voisey, P.W., Randall, C.J., and Larmond, E. 1975. Selection of an objective test of wiener texture by sensory analysis. Can. Inst. Food Sci. Technol. J. 8: 23.

Whiting, R.C., Benedict, R.C., Kunsch, C.A., and Woychik, J.H. 1984. Effect of sodium chloride levels in frankfurters on the growth of Clostridium sporogenes and Staphylococcus aureus. J. Food Sci. 49: 351.
Ms received 6/8/84; revised 11/19/84; accepted 11/30/84.

Development of a Frankfurter Analog
From Red Hake Surimi

E. M. BUCK and R. D. FAFARD

────────────ABSTRACT────────────

The development of an essentially fat-free, frankfurter analog from red hake surimi is described. In consumer tests against four commercial brands of frankfurters, the analog competed well with a chicken frankfurter, but neither chicken nor fish achieved the levels of acceptance of beef-containing products. Warner-Bratzler shear measurements revealed significant changes in texture in the fish and chicken frankfurters during a 21-day storage period at 2°C. The analog proved to have lower populations of certain microorganisms as compared to the other products tested. There was no spoilage detected in any of the products evaluated after 21 days at 2°C. The nutritional advantages of the analog are discussed.

INTRODUCTION

SEVERAL WORKERS have reported on the partial or complete replacement of red meats with fish muscle in a frankfurter formulation (Park et al., 1978; Howe, 1967; Spinelli et al., 1977; Mann, 1977; Webb et al., 1976). In all cases, unwashed fish flesh was used and in most cases the finished product contained relatively high percentages of fat. An undesirable soft texture associated with the experimental samples was frequently reported.

Holmquist et al. (1984) reported that red hake (*Urophycis chuss*), an underutilized species of fish found off the New England and Mid-Atlantic States, was suitable for the manufacture of surimi which could be stored frozen and ultimately manufactured into an acceptable Kamaboko. Surimi is prepared by washing minced fish flesh several times with cold water to remove water soluble proteins and other materials. It is then de-watered, mixed with cryoprotectants such as sugar, and tripolyphosphates, frozen and stored until used. To prepare Kamaboko, a white, bland, Surimi-gel/product prized by the Japanese for its rubbery texture, the thawed surimi is chopped with sufficient salt to extract myofibrillar protein, shaped, and heat-processed to form the protein gel.

There is an obvious market for meat or meat-like products with low fat contents and greater nutritional value. Since red hake has less than one-half of one percent fat and it has been demonstrated that it will produce an acceptable protein gel, it should be possible to produce a frankfurter analog from red hake which derives its texture from a protein gel rather than a fat emulsion. In addition, the extremely low fat product should have significantly fewer calories and more protein than a conventional frankfurter.

The objectives of this investigation were thus set as follows: (1) develop the processing parameters needed to produce an all fish frankfurter analog with the textural characteristics, flavor, and color of a conventional red meat product; (2) conduct storage and sensory acceptance studies of the analog.

MATERIALS & METHODS

Source of experimental material

Two hundred twenty-six kilograms of whole red hake (*Uro-*

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phycis chuss) were purchased from day boats operating out of Gloucester, MA in October, 1982. The iced fish were transported to the Dept. of Food Science & Nutrition in Amherst, MA and stored overnight in a 2°C cooler.

Preparation of surimi

The fish were hand filleted and skinned. A mince was prepared by grinding the fillets through a 6 mm plate in a Toledo Chopper (Model 5125). The mince was immediately washed according to the following procedure. An aliquot of mince weighing 2.25 kg was placed in a container with eight parts of an ice/distilled water mixture, stirred for 1 min and allowed to stand for approximately 20 min, or until the mince had settled. It was then decanted through a fine mesh screen (1 mm) and the washing repeated four more times. The fifth and final wash water contained 0.2% NaCl to aid in dewatering.

Several aliquots of washed mince were then wrapped in four layers of cheesecloth and placed in a Wabash Hydraulic Wine Press (model 30-24-SM) where they were subjected to 10,000 psi pressure for 20 min.

Following de-watering, the mince was placed in the pre-chilled bowl of a Toledo High Speed Mixer (Model TM20) and mixed for 5 min (medium speed) with 5.0% sucrose and 0.2% sodium tripolyphosphate (w/w). The resulting surimi was then divided into 1 kg portions and packed into opaque polyethylene bags so that as much air as possible was excluded. The bags were quickly cooled to 0°C in a blast freezer (−30°C) and stored at −18°C until used.

Manufacture of the frankfurter analog

The 1 kg bags of frozen surimi (5 months old) were thawed overnight in a 2°C cooler prior to further processing. The thawed surimi was placed in a 20L Stronia Silent Cutter (Model KS-20) and chopped for 2 min at high speed with 2.5% NaCl. The fish paste was kept below 15°C at all times by the application of bagged ice to the outside of the cutter bowl before and during chopping. At this point, the following ingredients were added: 0.24% white pepper, 0.32% garlic powder, 0.40% paprika, 0.15% coriander, 0.5% liquid smoke (Flavorite Lab, Memphis, TN.), 0.5% liquid beet juice concentrate (Beatrice Foods, Chicago, IL), 0.012% sodium nitrite and 0.055% sodium erythorbate. Chopping was then continued at high speed for an additional 8 min. The finished paste was transferred to a Vogt piston driven hand stuffer and stuffed into 24 mm cellulose casings (Union Carbide Co., Chicago, IL), which were then hand-linked every 12.5 cm. After standing at room temperature (25°C) for 1 hr, the links were heat processed by immersion in a 90°C water bath for 20 min. They were then cooled by immersion in a 2°C ice/water bath until an internal temperature of 30°C or lower was achieved, hung on smoke sticks, and held overnight in a 2°C cooler. The following day the casings were removed by hand-peeling (no steam) and the frankfurters were placed, 8 to a bag, in opaque polyethylene bags from which the air was evacuated with a mechanical vacuum pump. The sealed bags were stored in a 2°C cooler until tested.

Beginning with the filleting, all equipment used in processing was disinfected with Purina I-O Concentrate, a controlled iodine general purpose disinfectant.

Commercial frankfurters used for testing were purchased as fresh as possible from a local supermarket.

Shear measurements

Shear values were determined with a Warner-Bratzler Shear (Model 2000) equipped with a 10 lb dynamometer. Five links were randomly selected from each package, commercial or experimental, and each link was sheared twice perpendicular to its long axis,

dividing the link into equal thirds. All frankfurters were at room temperature when sheared. Shearing was conducted on day 1 of the storage period and weekly thereafter.

Consumer acceptability testing: In-house

Two separate trials were conducted in the Food Science Building using 5 groups of 20 randomly selected undergraduate students for each trial. The first trial was conducted on the day following manufacture of the experimental frankfurters and purchase of commercial samples. The second trial was conducted after these same samples had been stored for 21 days at 2°C. Each participant received samples of four commercial brands of frankfurters and a sample of the analog, individually and in succession. All samples had been held for 5 min in a 95°C water bath prior to presentation and were served in white plastic cups. A Latin Square design was used for both trials so that each sample was served in a different order to each group. Participants were asked to rate each sample using a 9-point hedonic scale where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely. Comments were also solicited.

Consumer acceptability testing: Out-of house

Two separate consumer evaluation studies were conducted on two consecutive days in an administration building on the Univ. of Massachusetts campus. In the first study a freshly prepared frankfurter analog was evaluated. In the second study, fresh commercial chicken frankfurters were used. In each trial, samples were warmed in a 95°C water bath and presented to 100 participants in a warmed frankfurter roll. Condiments such as mustard, relish, and catsup were available so that they could prepare their frankfurter sandwich as they wished. Participants were then asked to rate the product using a 9-point hedonic scale identical to that previously described. Comments were also solicited.

Microbiological tests

The following microbiological plate counts were performed at the beginning of the storage study and every 7 days thereafter for 4 wk: mesophilic, psychrotrophic, mesophilic anaerobic, coliform, and *C. perfringens*. Packages were randomly selected at each sampling interval and aseptically opened to permit transfer of sample to a sterile blender jar. Mesophilic and psychrotrophic aerobes were determined according to the method of Gilliland et al. (1976) *C. perfringens* was determined by the method of Duncan and Harmon (1976). Coliforms were determined by the procedures of Fishbein et al. (1976). Incubation times were as follows: mesophiles, 32°C for 48 hr; psychrotrophes, 5°C for 10 days; coliforms, 32°C for 24 hr; and *C. perfringens*, 35°C for 24 hr. Cultural procedures were used to enumerate mesophilic anerobes. The count was obtained using MPN tables (Hays and Lynt, 1976).

Chemical analysis

Moisture, crude fat, total nitrogen, ash, and sodium were determined in duplicate according to AOAC (1980) methods. Carbohydrate content was obtained by difference. The pH was determined by blending 10g of sample with 20 mL of distilled water for 1 min in a blender set at low speed. The pH of the resulting slurry was then read directly using a pH meter equipped with a suitable electrode.

Statistical analysis

The data were analyzed either by one-way or two-way analysis of variance. The Duncan range test was used to determine statistical significance.

RESULTS & DISCUSSION

THE EXPERIMENTAL frankfurter analogs were tested against four different kinds of commercial frankfurters deemed representative of their categories by an informal panel of individuals skilled in sampling foods. The four test brands were chosen from among 28 different commercial brands categorized according to meat content; all beef, beef-pork, turkey, or chicken.

The formulation used for the manufacture of the frankfurter analogs ultimately used in this investigation was determined by trial and error. Liquid beet juice concentrate was chosen as a colorant on the basis of favorable results reported earlier by Vereltzis and Buck (1984). There was a noticeable fading of the color of the analog during heat processing; however, this could be controlled, within limits, by adding more colorant and controlling processing time and temperature. The processing parameters ultimately used produced a color, after processing, which was in an acceptable range for frankfurters. After processing, the interior and exterior colors of the analog proved to be very stable as no noticeable changes were observed. This was also true of the commercial frankfurters.

Moisture, pH, and yield of mince and surimi

Average values for moisture content, pH, and yield of fish flesh in its various processed forms prior to manufacture of the analog are shown in Table 1. Approximately 37% of the minced flesh was lost during the washing and dewatering process. Most of this was due to loss of water-soluble proteins; however, it was felt that these losses could have been reduced if commercial continuous screw-type de-watering equipment had been used. The pH and moisture contents of the de-watered flesh were in the desired range for the manufacture of fish gel products (Suzuki, 1981).

Consumer evaluation

Results of the in-house consumer acceptability study are shown in Table 2. In trial 1, which was conducted at the beginning of the storage study, it may be seen that participants preferred the beef and beef-pork frankfurters to all others, and that the fish and chicken frankfurters were the least preferred ranking in between neither like nor dislike and dislike slightly. After 21 days of storage at 2°C, the beef, beef-pork, and turkey frankfurters dropped only slightly in their ratings; however, there were significant changes in the fish and chicken frankfurters relative to the other samples tested in that trial (P < 0.05).

The overall preference of consumers for beef or beef-pork frankfurters was not unexpected and has been reported before (Whiting and Jenkins, 1981). The lower rating of the fish frankfurter and the change in preference of the chicken frankfurter are possibly due to changes in texture, odor, and flavor during storage. For example, in trial 1, 9% of the participants commented on a fishy odor or flavor associated with the experimental frankfurter while

Table 1—Average values for moisture content, pH, and yield of unwashed mince, de-watered washed mince, and surimi

Treatment of flesh	Yield[a] %	Moisture content %	pH
Unwashed mince	32	82	6.80
De-watered, washed mince	20	80	6.85
Surimi	21	78	7.00

[a] Expressed as a percent of whole fish weight.

Table 2—Average scores[a] for consumer acceptability test (in-house) of different frankfurters

Trial no.[b]	Beef	Beef & Pork	Turkey	Fish	Chicken
1	7.1a	7.1a	6.1b	4.5c	4.3c
2	7.0a	6.9a	5.8b	3.3c	4.5d

[a] Nine-point hedonic scale (1=dislike extremely and 9=like extremely). Values in each row with the same letter are not significantly different (p<0.05).

[b] Trial 2 conducted after 21 days of storage at 2°C.

21% made similar comments in trial 2. The fish frankfurter was also described as having a rubbery teexture by 2% of the participants in trial 1 and 13% in trial 2.

The comments on texture are supported by the data on shear force which are shown in Fig. 1. The analog began the storage period with a shear value similar to that of the beef, beef-pork, and turkey frankfurters while the shear values for the chicken products were extremely low. After 21 days of storage, however, it may be seen that the analog progresses to a level above all the others while the chicken frankfurter shear values increase to a level similar to that of beef-containing products and stay there for the remainder of the storage period. Although participants did not specifically comment on the softness of the chicken frankfurter, its improvement in texture at 2°C may possibly have accounted for its increased level of acceptance. Likewise, the increase in textural properties (rubberiness) of the fish frankfurter during storage apparently contributed to its decreased level of acceptance.

The fishy taste or odor associated with the analog may have been due to the fact that the surimi used for manufacture of the analog had been stored for 5 months in a chest-type freezer operating at -18°C, but subject to frequent use and therefore serious temperature fluctuation. Also, the participants in the in-house consumer evaluation study were all aware of the fact that one of the frankfurters being tested was made with fish, and given the textural differences, they may have learned to unconciously identify it and therefore taste fish. Also, although participants did not examine samples simultaneously, they did receive them one after the other and there was probably some tendency to compare the least liked with the best liked.

In an attempt to remove bult-in bias and to test the analog under less rigorous conditions, it was decided to run a separate consumer evaluation test using freshly made frankfurters served under more normal conditions. The tests were conducted in a separate building where participants had no prior knowledge of the experimental product. Also, the analog was tested against a chicken frankfurter which the previous test had indicated was its most likely competitor. The results of this out-of-house evaluation are shown in Table 3.

Under the conditions of this evaluation, the analog competed quite favorably with a chicken frankfurter. It is interesting to note that both products received scores of "like moderately" which is considerably higher than either received when being tested against the beef-containing frankfurters. This suggests that food analogs do better in consumer evaluations when tested by temselves as differences probably become more obvious if its authentic counterpart is available for comparison. The freshness of both products also probably helped account for the higher scores. Not one of the 100 participants commented on any unusual flavor or odor being associated with the frankfurter analog, although 8% did comment that the chicken frankfurter was too soft. Consumers apparently prefer the texture of the analog when fresh and the texture of a chicken frankfurter after at least 1 or 2 wk of refrigerator storage.

Microbiological evaluation

Variations in numbers of aerobic psychrotrophes, aerobic mesophiles, and anaerobic mesophiles are shown in Fig. 2, 3, and 4 respectively. The counts for these organisms were lower initially in the analog and remained lower throughout the 3-wk storage period. This probably was due in part to the water bath processing method used and to the sanitizing procedures employed during manufacture of the surimi and the analog. The numbers of all of these organisms increased during storage but none had reached undesirable levels by the end of 21 days. Zottola (1972) reported that 1.5×10 microorganisms/gram (APC) are required to detect spoilage in frankfurters. After 21 days at 2°C, the chicken frankfurter had the highest population with an APC/g of 7.7×10 (Fig. 3), yet there was no sensory evidence of spoilage in any of the samples.

Neither *C. perfringens* nor coliform bacteria were recovered during the course of the investigation.

Proximate analysis

The nutritional advantages of the frankfurter analog may be seen in Table 4. The analog contained essentially no fat (0.09%) while conventional frankfurters contained varying amounts of fat up to 33%. Due to its low fat content, the analog contained only 26 – 46% of the calories of the other products tested. It also contained up to 34% more protein than the other products tested and approximately 30% less sodium. Although water obviously replaces much of the fat in the gel system employed, the analog still easily meets USDA requirements that percent moisture be no higher than four times the percent protein plus ten percent.

CONCLUSIONS

IT HAS BEEN DEMONSTRATED that it is possible to produce an all fish, essentially fat-free, frankfurter analog with an acceptable texture by employing a manufacturing system based on the development of a protein gel. Although
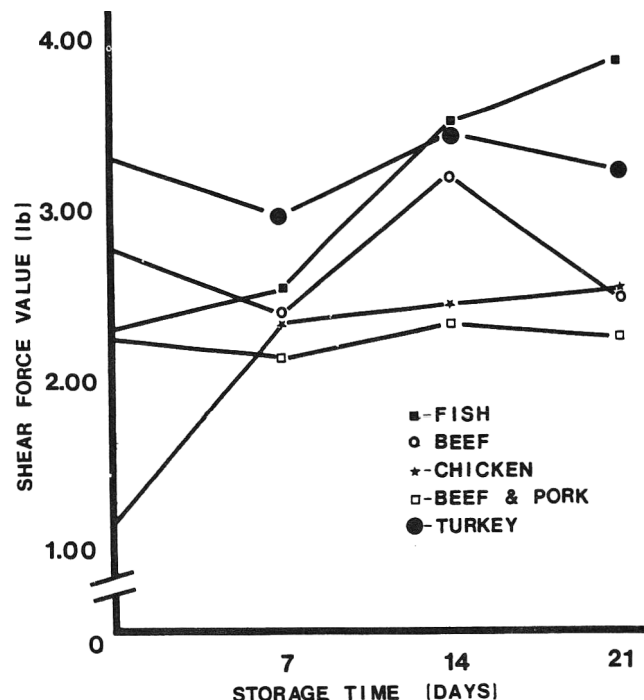
Fig. 1—Average shear force values for the analog and four commercial frankfurters during storage at 2°C.

Table 3—Average scores[a] for consumer evaluation study of fish and chicken frankfurters

Frankfurter type	Evaluation score
Fish	7.0a
Chicken	6.9a

[a] Values in each column with the same letter are not significantly different. (p < 0.01).

the product, as formulated, did not achieve levels of acceptance in consumer tests equal to those of beef-containing products, it did compete well with a chicken frankfurter, especially if fresh surimi was used in its manufacture.

It is believed that the problems associated with the development of a fishy flavor or odor during storage are related to storage time and temperature of the surimi used in the manufacture of the analog. Further investigation is needed in this area.

The increase in rubberiness during refrigerator storage as emphasized by consumer comments and shear measurements, although not considered serious by the authors, may be a function of the species of fish used and also needs additional study.

The nutritional advantages of the analog as formulated should make it of interest to a large segment of the population. The ability of the analog to compete with conventional beef containing products could conceivably be increased by adding fat to the formulation, either animal or vegetable, and meat flavoring materials.

REFERENCES

AOAC. 1980. "Official Method of Analysis." 13th ed. Association of Official Analytical Chemists. Washington, D.C.

Duncan, L.C. and Harmon, S.M. 1976. Clostridium Perfringens. In "Compendium of Methods for the Microbiological Examination of Foods," (Ed.) Speck, M.L. American Public Health Association, Washington, DC.

Fishbein, M., Mehlman, I.J., Chugg, L., and Olson, J.C. 1976. Coliforms, fecal coliforms, E. Coli, and enteropathogenic E. Coli. In "Compendium of Methods for the Microbiological Examination of Foods." (Ed.) Speck, M.L. American Public Health Association, Washington, DC.

Fig. 2—Variations in plate counts of aerobic psychrotrophs in chicken, beef, and fish frankfurters during storage at 2°C. Plates incubated 10 days at 5°C.

Table 4—Proximate analysis of the experimental frankfurter analog and the four commercial frankfurters against which it was tested

	Fish	Beef	Beef & Pork	Chicken	Turkey
Protein (%)	17.31	11.36	11.62	13.03	12.32
Fat (%)	0.09	30.03	32.97	15.30	22.21
Carbohydrate (%)	5.13	0.00	0.00	2.11	0.00
Moisture (%)	74.93	53.37	51.86	64.41	60.46
Sodium (mg/100g)	671	835	1153	847	976
Ash (%)	1.93	2.61	3.29	3.22	3.51
Calories/100g	90	315	343	195	249
% of calories attributed to protein	77	14	13	26	20
% of calories attributed to fat	0.9	86	87	70	80

Fig. 3—Variations in plate counts of aerobic mesophils in chicken, beef, and fish frankfurters at 2°C. Plates incubated 48 hr at 32°C.
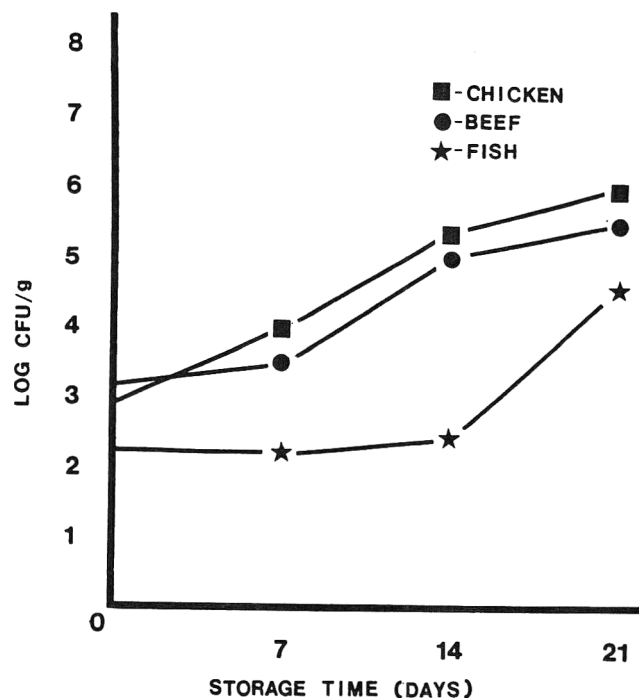
Fig. 4—Variations in plate counts of anaerobic mesophils in chicken, beef, and fish frankfurters at 2°C. Plates incubated 48 hr at 32°C.

Lactic Fermentation of Ground Soybean for Use in Imitation Cream Cheese Products

CONSTANCE J. HOFMANN and WAYNE E. MARSHALL

————— ABSTRACT —————

Ground soybean fermented with *Lactobacillus casei* ssp. *rhamnosus* (ATCC 39595) was found to contain diacetyl and acetylmethylcarbinol. The fermented ground soybean showed significantly improved flavor and texture compared to nonfermented material when used to replace nonfat dry milk in an imitation cream cheese product. The flavor improvement was due principally to the presence of diacetyl but the factor(s) responsible for texture improvement was not identified. Diacetyl production by *L. casei* was enhanced by the addition of sodium acetate or sodium citrate to the ground soybean suspension before fermentation.

INTRODUCTION

EDIBLE PRODUCTS derived from soybeans have been slow to gain acceptance in Western culture. Only the extracted oil has acquired commercial importance. The less refined soy products such as ground soybean and soymilk have been considered particularly unacceptable in terms of flavor and texture. Recent attention has been focused on improving the flavor and texture of soymilk using lactic acid bacteria (Mital and Steinkraus, 1979; Patel et al., 1980), in order to develop yogurt-like products. The principal flavor- and texture-modifying component in these fermentations was lactic acid, which was an end-product of the bacterial metabolism of soy sugars.

In contrast to the sour, astringent flavors produced by the accumulation of lactic acid, few reported attempts have been made to produce flavor and texture associated with cultured buttermilk or cream cheese products using ground soybean or soymilk as substrates. In these products, made traditionally from bovine milk, the characteristic flavors arise primarily from bacterial production of small, volatile compounds such as diacetyl, acetic and propionic acids (Hammer and Babel, 1943). Gehrke and Weiser (1948) observed production of diacetyl and the related compound acetylmethylcarbinol (AMC) in soymilk using *Streptococcus* species known to produce these compounds in bovine milk. In addition to *Streptococcus* species, certain species of Lactobacillus such as *L. casei*, have been reported to produce diacetyl and AMC in either bovine milk (Bassette et al., 1967; Keenan and Lindsay, 1968) or in broth medium (Branen and Keenan, 1971; Benito de Cardenas et al., 1980, 1983; Hegazi and Abo-Elnaga, 1980). Two studies have used *L. casei* for soymilk fermentation (Angeles and Marth 1971a, b, c, d; Yamanaka and Furukawa, 1970). Angeles and Marth measured bacterial growth, acid production, and lipolytic and proteolytic activity while Yamanaka and Furukawa measured acid production and curd hardness in skimmilk-soymilk combinations. We are not aware of any published report which describes the use of *L. casei* for diacetyl and AMC production using soy as a substrate.

The purpose of this investigation was to improve the food use of ground soybean by imparting flavor and texture

compatible with cream cheese products. In this regard, a fermentation of ground soybean was performed using a selected strain of *L. casei* ssp. *rhamnosus*. Traditional diacetyl- and AMC-producing cultures were screened but did not produce significant levels of flavor compounds within the same time period as *L. casei*.

MATERIALS & METHODS

Preparation of ground soybean

Clean, dry soybeans of the Amsoy variety were fed into the hopper of a Jabez-Burns mill at the rate of 1.4 kg/min along with sufficient hot water (86°C) to produce a water:bean ratio of 10:1. Sufficient calcium hydroxide of a 30% (w/v) suspension was metered into the hopper to give a ground slurry pH of 8.7 – 8.8 in order to promote flavor improvement of the ground soybean. The temperature of the slurry leaving the mill was 80°C. The slurry was immediately fed into an Urschel comitrol with a microcut (216 T) head. The finer grinding raised the temperature of the slurry to 86°C where it was held for 1 – 2 min. The slurry was then brought to pH 6.5 with 85% phosphoric acid, in order to optimize the pH for subsequent lactic fermentation. Finally, the slurry was pasteurized at 116°C for 15 sec, and spray dried. The proximate analysis of spray-dried, ground soybean is given in Table 1.

Microorganism

The microorganism used for the fermentations was *Lactobacillus casei* ssp. *rhamnosus* (ATCC 39595). The microorganism was originally a gift from Dr. F.M. Strong, Univ. of Minnesota, but was identified at Kraft, Research & Development as subspecies *rhamnosus*. A culture of the microorganism was deposited with the American Type Culture Collection where it was given the identification ATCC 39595.

The culture was propagated in a lactobacillus starter medium which consisted of whey permeate, soy peptone, glucose, yeast extract, Tween 80, salts, and water. The cells were concentrated to a cell density of 1×10^{12} cells/g, placed in foil pouches, frozen in liquid nitrogen, and stored at −90°C. The foil pouches were thawed in warm water before use.

Spray-dried ground soybean was reconstituted with water to 10% (w/v) solids and pasteurized at 70°C for 20 min. The pasteurized sample was cooled to 32°C and, if required, solid sodium acetate or sodium citrate was added to the desired concentration. After

Table 1—Proximate analysis[a] of ground soybean

Component	Percent of component
Protein	40.5
Fat	24.6
Crude fiber	4.3
Ash	5.6
Sugars (Total)	10.9
Moisture	3.2
Complex carbohydrates[b]	14.1
Glucose	0.06
Lactose	0.00
Acetate (as acetic acid)	0.18
Citrate (as citric acid)	0.58

[a] Values were determined by standard AOAC methods and are given on a dry weight basis, except moisture.
[b] Calculated by difference.

dissolution of the acetate or citrate, the *L. casei* culture was added to produce the desired inoculum level. Optimum values for initial fermentation pH, temperature, and time were determined. Based on these findings, the fermentation was carried out at 32°C for 4 hr with the initial pH adjusted to 6.5. After 4 hr, the final, viable cell count generally increased twofold from the initial, viable cell count and the final pH decreased 0.1 – 0.2 pH units, which indicated some growth and some glycolysis occurred during flavor volatile production. Finally, the fermented product was pasteurized at 70°C for 20 min and spray-dried. Nonfermented ground soybean was treated in a similar manner, except no microbial culture was added.

Determination of diacetyl and AMC

Diacetyl, AMC, and other neutral volatiles present in ground soybean were quantified by a gas chromatographic procedure (Jackson, 1981). Diacetyl and AMC were quantified by adding known amounts of these compounds to the nonfermented ground soybean samples and measuring the resultant peak areas. In this manner, a standard curve of peak area versus amount of compound was generated. The recovery of both diacetyl and AMC was greater than 90% after only one purge. Positive identification of all neutral volatiles was made by standard gas chromatography/ mass spectroscopy techniques.

Imitation cream cheese product

The imitation cream cheese product consisted of water, vegetable oil, nonfat dry milk, salts, emulsifiers, stabilizers, and acidulant. In a typical application, fermented or nonfermented ground soybean, which comprised 4.2% of the finished product, replaced 33% of the nonfat dry milk. The vegetable oil was heated to 57°C and the ground soybean added to it. Water was heated to 43°C and nonfat dry milk, salts, emulsifiers and stabilizers were dispersed in the water. The dispersion was heated to 57°C and the soy-oil blend added to it. Lactic acid was added to produce a product pH of 4.6 – 4.8. The imitation cream cheese was then homogenized in two stages at 2100/500 psi and poured into containers and cooled. The imitation cream cheese, without ground soybean, was judged to be bland in flavor and smooth in texture so the addition of fermented or nonfermented ground soybean would impart its own characteristic flavor and texture.

Sensory evaluation

The sensory evaluation procedure consisted of a preference/ attribute test design with balanced sequential presentation of samples and use of nine-point intensity and hedonic scales. Samples were served as 0.5 cm x 2.5 cm rectangles at a sample temperature of 7 – 10°C. Panelists were instructed to eat an unsalted cracker and to rinse their mouth between samples. The interval between samples was 1 min and fluorescent lighting was used in the panel booths.

Two different sensory panels were used. One panel (8-12 trained members) evaluated the imitation cream cheese for cream cheese flavor intensity on a 1 to 9 scale where 1 = weak, 5 = moderate, and 9 = strong. The other panel (30 untrained members who used and liked cream cheese) evaluated the imitation cream cheese for product appearance, texture, cream cheese flavor, flavor, and acceptability. Their ratings were based on a 1 to 9 hedonic scale where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely, except the rating for cream cheese flavor was 1 = weak, 5 = moderate, and 9 = strong. Mean hedonic or flavor intensity scores were tabulated for each attribute and a 2-way analysis of variance was used to determine significance between mean scores.

RESULTS & DISCUSSION

Characterization of neutral volatiles

The neutral volatile analysis of nonfermented ground soybean (Fig. 1A) shows four major peaks which were identified by mass spectroscopy as acetone, n-pentanal, n-pentane, and n-hexanal in order of increasing elution time. These are typical neutral volatiles found in soy products and are the result of lipoxygenase activity on unsaturated fatty acids found in the soybean oil fraction (Wilkens et al., 1967). These volatiles may form in the intact bean but most likely occur during grinding of the bean and before enzyme inactivation by hot water (Nelson et al., 1971). The neutral volatile profile of fermented ground soybean (Fig. 1B) shows two additional peaks which were identified as diacetyl (peak 4) and acetylmethylcarbinol (peak 5). This chromatogram is also noteworthy in that the volatile n-pentanal (peak 2', Fig. 1A) disappeared and acetone, n-pentane, and n-hexanal were reduced in concentration. Fermentation of ground soybean by *L. casei* apparently reduces the levels of these volatiles, possibly by disrupting the binding of these compounds to soy protein. Thus, *L. casei* ssp. *rhamnosus* (ATCC 39595) is capable of producing diacetyl and AMC in ground soybean.

Production of diacetyl and AMC by *Lactobacillus casei*

The production of diacetyl in ground soybean after a 4-hr fermentation increased linearly with increased initial cell concentration over the range 5×10^6 to 1×10^9 cells/mL (Fig. 2). There appeared to be a lag in the production of AMC until a cell concentration of 5×10^7 cells/mL was reached and then the compound was produced at a linear rate. However, the rate of AMC production was less than the rate of diacetyl production and the amount of diacetyl was 4 to 14 times greater than AMC in the range of cell concentrations given in Fig. 2.
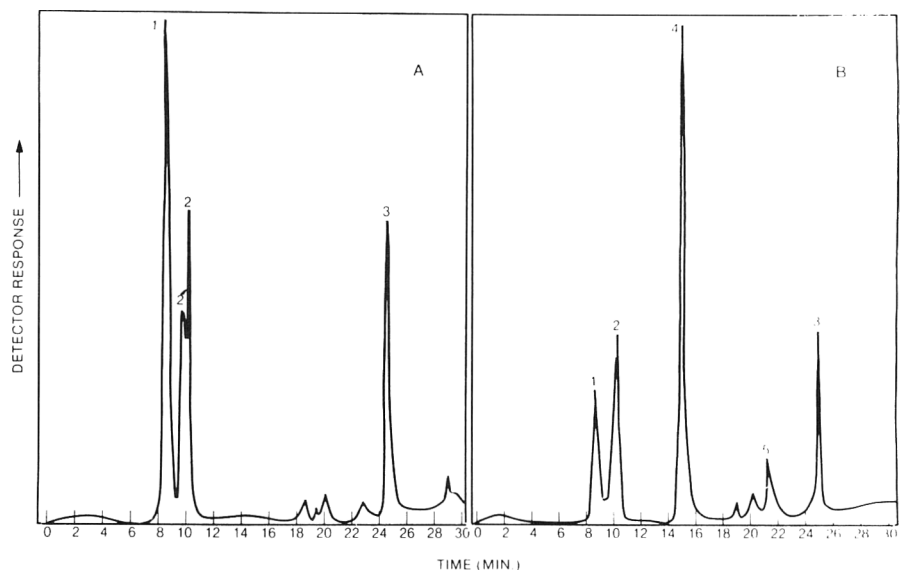
Fig. 1—Gas chromatographic elution profiles of spray dried, ground soybean: (A) Nonfermented, (B) Fermented. Elution peaks were identified as 1 = acetone; 2' = n-pentanal; 2 = n-pentane; 3 = n-hexanal; 4 = diacetyl; 5 = AMC. Fermentation conditions were: temperature = 32°C; initial pH = 6.5; time = 4 hr; initial cell concentration = 1 x 10^8 cells/mL.

The importance of flavor perception as a function of diacetyl concentration was examined. Ground soybean was fermented at either different pH values but at constant temperature or at different temperatures but at constant pH in order to change the diacetyl concentrations of the samples. These samples were incorporated into imitation cream cheese and evaluated for cream cheese flavor intensity by the trained taste panel. The cream cheese flavor intensity score was plotted versus the diacetyl concentration for each sample. The results are shown in Fig. 3. The data can be represented by the straight line $Y = 0.092 X + 3.33$ and the correlation coefficient $R = 0.76$. The probability of a linear relationship with $R = 0.76$ is greater than 99%, given 11 degrees of freedom. Therefore, diacetyl concentration is directly related to the perception of cream cheese flavor in the imitation cream cheese product.

Sensory evaluation of imitation cream cheese products made with fermented and nonfermented ground soybean

Many of the imitation cream cheese products evaluated for cream cheese flavor intensity had flavor intensity scores in the moderate to strong (5 – 9) range (Fig. 3). To determine if product made with fermented ground soybean was significantly better than product made with nonfermented ground soybean, not only in terms of cream cheese flavor intensity but in terms of other product attributes as well, a panel consisting of employees who used and liked cream cheese was utilized. The panelists were asked to rate the products with respect to five different attributes, including flavor (Table 2). In every category except appearance, the sample with fermented ground soybean was significantly preferred to the product with nonfermented ground soybean. The significant differences in flavor and cream cheese flavor scores show that the perception of cream cheese flavor is a positive attribute in the test product, and probably one that weighs heavily in total flavor perception.
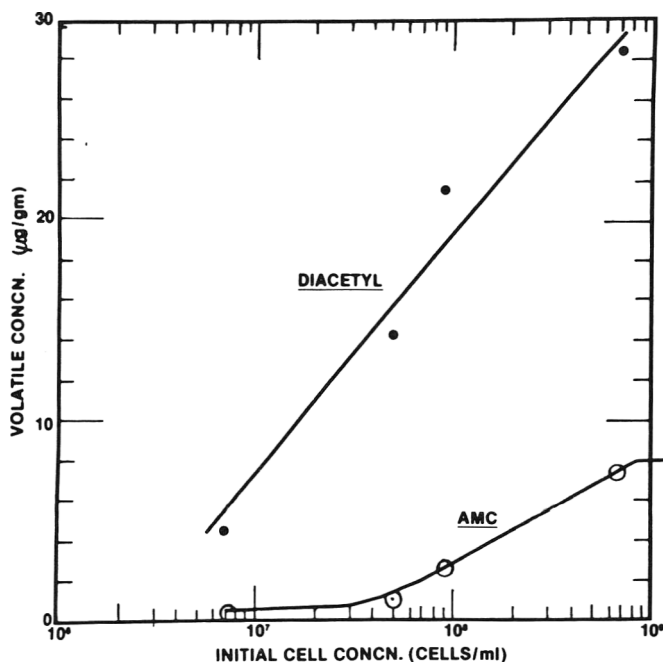
The reason for the significant difference in texture between products was not identified. Lipolysis, and/or proteolysis during fermentation could alter the texture of the ground soybean material. Angeles and Marth (1971c, d) reported lipolytic but not proteolytic activity using *L. casei* in the fermentation of soymilk. However, significant lipolysis was observed only after 14 days. The significant difference in acceptability score points out the consistency by which the panelists rated product flavor and texture. Product flavor and texture were apparently weighted more than product appearance in reaching an overall acceptance.

Improved production of diacetyl and AMC from *Lactobacillus casei*

Fermentation of ground soybean was shown to significantly improve its use in an imitation cream cheese product (Table 2). Since product acceptance was based, in part, on flavor acceptance, which is directly related to diacetyl concentration (Fig. 3), methods of increasing the diacetyl level in fermented soy without increasing the initial cell concentration were investigated. To produce acceptable product in terms of cream cheese flavor, i.e. flavor intensity score = 5.0, a diacetyl concentration of about 20 $\mu g/g$ sample is needed (Fig. 3). Twenty $\mu g/g$ corresponds to a cell density of about 10^8 cells/mL (Fig. 2). This is a large inoculum, and an inoculum of $10^6 - 10^7$ cells/mL would be preferred because of potential cost savings.

Studies by several different laboratories (Branen and Keenan, 1971; Benito de Cardenas, 1980; Hegazi and Abo-Elnaga, 1980) have observed increased production of both diacetyl and AMC in *L. casei* cultures with the addition of pyruvate, citrate, or acetate to the fermentation medium. Based on these reports, ground soybean was fermented in the presence of pyruvate, citrate, or acetate. Ground soybean was also fermented in the presence of glucose to determine the effect of increased glycolytic activity (glucose to lactic acid) on the production of diacetyl and AMC (Table 3). The addition of sodium acetate or sodium citrate to a concentration of 0.15% of the ground soybean suspension caused *L. casei* to produce more diacetyl and AMC than the control fermentation. Slightly more diacetyl but less AMC was produced in the presence of acetate when compared to citrate. *L. casei* produced 46% and 42% more diacetyl with added acetate and added citrate, respectively.

Fig. 2–Volatile concentrations in spray dried, ground soybean fermented at different initial cell concentrations. Volatile concentrations are the difference in concentration of diacetyl and AMC between time = 4 hr and time = 0 hr samples. Initial cell concentration is given as the number of viable cells per mL of a 10% (w/w) ground soybean suspension. Fermentation conditions were: temperature = 32°C; initial pH = 6.5; time = 4 hr.

Fig. 3–Cream cheese flavor intensity score versus the diacetyl content of fermented, spray dried ground soybean used in the imitation products. Flavor intensity scores based on a 1 to 9 rating system where 1 = weak; 5 = moderate; 9 = strong. Diacetyl content is the difference in concentrations between time = 4 hr and time = 0 hr samples.

In contrast to these results, *L. casei* produced 40% and 44% less diacetyl in the presence of 0.15% sodium pyruvate or 1.0% glucose, respectively, when compared to the control fermentation. Less AMC was also observed under these conditions. The reduced levels of diacetyl and AMC with added pyruvate may be partially explained from the observations of Hegazi and Abo-Elnaga (1980). They observed the production of diacetyl and AMC from *L. casei* using a broth medium supplemented with pyruvate or pyruvate plus glucose. The production of diacetyl and AMC was greatly diminished in the medium with no glucose. Therefore, glycolysis was apparently necessary to counteract the pyruvate inhibition of flavor volatile production.

Gound soybean contains only 0.06% glucose and no lactose (Table 1), so easily fermented sugars are not available to *L. casei* in this medium. Ground soybean has abundant sucrose, but *L. casei* apparently does not metabolize sucrose (Angeles and Marth, 1971a; Bucker et al., 1979). Therefore, glycolysis in the presence of pyruvate does not occur in the present case and flavor volatile production would not be expected to increase. The result with 1.0% glucose (Table 3) suggests that activation of the glycolytic pathway in *L. casei* inhibits the metabolic pathway which produces diacetyl and AMC in a soy suspension.

Ground soybean contains 0.18% acetate and 0.58% citrate (Table 1), compared to 1.6% citrate (dry weight basis) found in bovine milk. Since these compounds can stimulate diacetyl and AMC production in *L. casei,* these concentrations (0.02% acetate and 0.06% citrate in the 10% suspension) may be sufficient to account for the levels of diacetyl and AMC normally observed in fermented ground soybean. The results from Table 3 show that the concentrations of diacetyl and AMC can be increased by the addition of sodium acetate or sodium citrate to ground soybean material.

Determination of optimum concentrations of sodium acetate and sodium citrate for diacetyl production

Ground soybean was fermented at four different concentrations of sodium acetate and three different levels of sodium citrate. The amount of diacetyl was determined for each sample and the samples incorporated into imitation cream cheese, which was then rated for cream cheese flavor intensity (Table 4). Sodium acetate added at 0.30% and at 0.60% concentrations resulted in cream cheese products with similar hedonic ratings. These two ratings were the highest of the four samples but the 0.30% sample was the only one significantly different from the sample with no acetate added and also had the highest concentration of diacetyl. The 0.30% sample gave the best overall results.

The results with sodium citrate were more clearcut. The cream cheese made from material fermented in the presence of 0.15% sodium citrate was clearly the best sample. It had the highest hedonic rating, greatest concentration of diacetyl, and its hedonic score was the most significantly different from the sample with no citrate added. Therefore, based on the results presented in Table 4, concentrations of 0.30% sodium acetate or 0.15% sodium citrate are the optima for the fermentation of ground soybean with *L. casei* ssp. *rhamnosus.* Using these optimum concentrations, the amount of diacetyl generated is increased 37% and 51% over control values in the presence of sodium acetate or sodium citrate, respectively.

It was determined earlier that an initial *L. casei* concentration of about 10^8 cells/mL was necessary to give an acceptable hedonic rating of 5.0 for cream cheese flavor intensity in the imitation cream cheese product. Using ground soybean fermented with optimum concentrations of acetate or citrate, only 10^7 cells/mL were required to produce fermented ground soybean with a diacetyl concentration of 20 $\mu g/g$, and an acceptable hedonic flavor rating of 5.0 (data not shown). The initial cell inoculum requirement was thus reduced by a factor of 10. Supplementation

Table 2—Sensory evaluation of imitation cream cheese made with fermented and nonfermented ground soybean

Product attribute	Mean scores[a]		Significant[b] difference in mean scores
	Fermented	Nonfermented	
Appearance	6.3	6.5	NSD[c]
Texture	5.4	4.6	<0.05
Cream cheese flavor	4.1	3.4	<0.05
Flavor	4.3	3.3	<0.01
Acceptability	4.2	3.3	<0.01

[a] Based on a hednonic rating scale of 1 to 9 for Appearance, Texture, Flavor, and Acceptability where 1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely; based on an intensity rating scale for cream cheese flavor where 1 = weak; 5 = moderate; 9 = strong.
[b] The difference in mean scores between fermented and nonfermented samples for each attribute was tested for significance using a 2-way analysis of variance.
[c] NSD = no significant difference.

Table 3—Diacetyl and AMC content of ground soybean fermented in the presence of acetate, citrate, pyruvate or glucose

Treatment[a]	Diacetyl[b] ($\mu g/g$)	AMC[b] ($\mu g/g$)
None	30.1	2.6
Sodium acetate (0.15%)	44.0	3.2
Sodium citrate (0.15%)	42.7	4.0
Sodium pyruvate (0.15%)	18.2	2.0
Glucose (1.0%)	17.0	2.3

[a] Percent of treatment is given as weight of salt or glucose per weight of a 10%(w/w) ground soybean suspension.
[b] Diacetyl and AMC concentrations were determined on the fermented spray dried powder, and represent the difference in concentrations between time = 4 hr and time = 0 hr samples. Fermentation conditions were: temperature = 32°C; initial pH = 6.5; time = 4 hr; initial cell concentration = 1 x 10^8 cells/mL.

Table 4—Effect of sodium acetate or sodium citrate on diacetyl production and cream cheese flavor intensity

Percent added[a]	Diacetyl[b] ($\mu g/g$)	Mean[c] score	Significant[d] difference in mean scores
Sodium Acetate			
0.00	31.7	5.3	—
0.15	42.4	5.6	NSD[e]
0.30	43.5	6.3	<0.05
0.60	39.2	6.4	NSD
1.00	30.9	5.1	NSD
Sodium Citrate			
0.00	31.7	5.3	—
0.15	47.8	6.7	<0.01
0.30	41.9	6.4	<0.05
0.60	36.9	5.9	NSD

[a] Percent added is given as weight of additive per weight of a 10% (w/w) ground soybean suspension.
[b] Diacetyl determined on the fermented, spray-dried powder, and represent the different in concentrations between time = 4 hr and time = 0 hr samples. Fermentation conditions were: temperature = 32°C; initial pH = 6.5; time = 4 hr; initial cell concentration = 1 x 10^8 cells/mL.
[c] Based on a cream cheese flavor intensity rating scale of 1 to 9 where: 1 = weak; 5 = moderate; 9 = strong.
[d] The difference in mean scores between the sample with no salts and the samples with different percent of sodium acetate or sodium citrate added was tested for significance using a 2-way analysis of variance.
[e] NSD = no significant difference.

with these salts is another method of enhancing cream cheese flavor in addition to using high inoculum levels.

SUMMARY

FERMENTATION of ground soybean with *L. casei* ssp. *rhamnosus* (ATCC 39595) changed the flavor and texture of the fermented material. The fermented ground soybean was perceived as having cream cheese compatible flavor and texture when used as a nonfat dry milk substitute in an imitation cream cheese. The principal flavor component was diacetyl. Diacetyl concentration and, hence, perceived cream cheese flavor could be improved by the addition of sodium acetate or sodium citrate to the fermentation. The addition of these salts reduced the amount of microbial culture required to produce acceptable product. The use of fermented ground soybean could be considered a less costly alternative to fermented milk products where cream cheese compatible flavor and texture are required.

REFERENCES

Angeles, A.G. and Marth, E.H. 1971a. Growth and activity of lactic-acid bacteria in soymilk. 1. Growth and acid production. J. Milk Food Technol. 34: 30.

Angeles, A.G. and Marth, E.H. 1971b. Growth and activity of lactic-acid bacteria in soymilk. 2. Heat treatment of soymilk and culture activity. J. Milk Food Technol. 34: 63.

Angeles, A.G. and Marth, E.H. 1971c. Growth and activity of lactic-acid bacteria in soymilk. 3. Lipolytic activity. J. Milk Food Technol. 34: 69.

Angeles, A.G. and Marth, E.H. 1971d. Growth and activity of lactic-acid bacteria in soymilk. 4. Proteolytic activity. J. Milk Food Technol. 34: 124.

Bassette, R., Bawdon, R.E., and Claydon, T.J. 1967. Production of volatile materials in milk by some species of bacteria. J. Dairy Sci. 50: 167.

Benito de Cardenas, I.L., Pesce de Ruiz Holgado, A.A., and Oliver, G. 1980. The effect of pyruvate on the production of flavor compounds by Lactobacillus casei ATCC 7469. Milchwiss. 35: 296.

Benito de Cardenas, I.L., Ledesma, O., Pesce de Ruiz Holgado, A.A., and Oliver, G. 1983. Diacetyl and acetoin production by lactobacilli in a synthetic medium. Milchwiss. 38: 218.

Branen, A.L. and Keenan, T.W. 1971. Diacetyl and acetoin production by Lactobacillus casei. Appl. Microbiol. 22: 517.

Bucker, E.R. Jr., Mitchell, J.H. Jr., and Johnson, M.G. 1979. Lactic fermentation of peanut milk. J. Food Sci. 44: 1534.

Gehrke, C.W. and Weiser, H.H. 1948. A comparative study of the biochemical activity of Streptococcus lactis, Streptococcus citrovorus, and Streptococcus paracitrovorus when grown on cow's milk and soybean milk. J. Dairy Sci. 31: 213.

Hammer, B.W. and Babel, F.J. 1943. Bacteriology of butter cultures: A review. J. Dairy Sci. 26: 83.

Hegazi, F.Z. and Abo-Elnaga, I.G. 1980. Production of acetoin and diacetyl by lactic acid bacteria in skimmed milk with added citrate and pyruvate. Z. Lebensm. Unters. Forsch. 171: 367.

Jackson, H.W. 1981. Techniques for flavor and odor evaluation of soy oil. J. Am. Oil Chem. Soc. 58: 227.

Keenan, T.W. and Lindsay, R.C. 1968. Diacetyl production and utilization by Lactobacillus species. J. Dairy Sci. 51: 188.

Mital, B.K. and Steinkraus, K.H. 1979. Fermentation of soy milk by lactic acid bacteria. A review. J. Food Protect. 42: 895.

Nelson, A.I., Wei, L.S., and Steinberg, M.P. 1971. Food products from whole soybeans. Soybean Digest 31: 32.

Patel, A.A., Waghmare, W.M., and Gupta, S.K. 1980. Lactic fermentation of soymilk — A review. Process Biochem. 15: 9.

Wilkens, W.F., Mattick, L.R., and Hand, D.B. 1967. Effect of processing method on oxidative off-flavors of soybean milk. Food Technol. 21: 1630.

Yamanaka, Y. and Furukawa, N. 1970. Studies on utilization of soybean protein for food processing. 2. Influence of soymilk added to skim milk on the acidity and the hardness of curd produced by lactic acid bacteria for dairy use. J. Food Sci. Technol. Tokyo. 17: 456.

Gilliland, S.E., Busta, F.F., Brinda, J.J., and Campbell, J.E. 1976. Aerobic Plate Count. In "Compendium of Methods for the Microbiological Examination of Foods" (Ed.) Speck, M.L. American Public Health Association, Washington, DC.

Hays, G.L. and Lynt, R.K. 1976. Mesophilic Sporeforming Anaerobes. In "Compendium of Methods for the Microbiological Examination of Foods" (Ed.) Speck, M.L. American Public Health Association, Washington, DC.

Holmquist, J.F., Buck, E.M., and Hultin, H.O. 1984. Properties of Kamaboko made from Red Hake (Urophycis Chuss) fillets, mince, or surimi. J. Food Sci. 49: 192.

Howe, J.L. 1972. Utilization of by-product fish racks. Master's project. Univ. of Massachusetts at Amherst.

Mann, J.D. 1977. Fish products from neglected species. Food Eng. 49: 106.

Park, E.Y., Brekke, C.J., and Branen, A.L. 1978. Use of Pacific hake in a frankfurter formulation. J. Food Sci. 43: 1675.

Spinelli, J., Koury, B., Groninger, H., and Miller, R. 1977. Expanded uses for fish protein from underutilized species. Food Technol. 15: 468.

Suzuki, T. 1981. "Fish and Krill Protein Processing Technology." Applied Science Publishers, Ltd. London.

Vereltzis, K.P. and Buck, E.M. 1984. Color stability and sensory attributes of chicken frankfurters made with betalains and potassium sorbate vs. sodium nitrite. J. Food Prot. 47: 41.

Webb, N.B., Hardy, E.R., Giddings, G.G., and Howell, A.J. 1976. Influence of mechanical separation upon proximate composition, functional properties and textural characteristics of Atlantic croaker muscle tissue. J. Food Sci. 41: 1277.

Whiting, R.C. and Jenkins, R.K. 1981. Comparison of rabbit, beef, and chicken meats for functional properties and frankfurter processing. J. Food Sci. 46: 1693.

Zottola, E.A. 1972. "Introduction to Meat Microbiology." AMI Center for Continuing Education. American Meat Institute. Chicago, IL.

Limonoid Debittering of Citrus Juice Sera By Immobilized Cells of *Corynebacterium fascians*

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──────── ABSTRACT ────────

Corynebacterium fascians cells capable of metabolizing limonoids were prepared conveniently with inexpensive carbon sources such as fructose, galactose and citric acid. Cells thus obtained were immobilized in acrylamide gel and used in a biological debittering process previously developed. The process significantly reduced limonin and nomilin contents of citrus juice sera. It was particularly effective toward the reduction of nomilin. The debittering treatment did not have adverse effects on the composition of other citrus constituents such as citric, malic, ascorbic acids, fructose, glucose and sucrose.

INTRODUCTION

BITTERNESS DUE TO LIMONOIDS such as limonin and nomilin in certain citrus juices is one of the major problems of the citrus industry world-wide and has significant economic impact. One of our approaches to solve this problem is to develop a biological process to reduce bitter limonoids of citrus juices.

During the course of biochemical studies of limonoids, we have isolated from soil five species of bacteria capable of metabolizing limonoids (Hasegawa et al., 1972, 1974a, 1983b; Hasegawa and Kim, 1975; Hasegawa and King, 1983) and have shown the presence of five metabolic pathways of limonoids in bacteria (Hasegawa et al., 1972, 1974b, 1983a, 1984; Hasegawa and Bennett, 1983). Based on these studies we have developed a biological process that uses immobilized bacterial cells for the reduction of limonoids in citrus juice sera (Hasegawa et al., 1982). Two species of bacteria, *Arthrobacter globiformis* and *Arthrobacter globiformis* II have been used for this purpose (Hasegawa et al., 1982, 1983b; Hasegawa and Pelton, 1983). *A. globiformis* cells immobilized in acrylamide gel converted limonin and nomilin of citrus juice to nonbitter 17-dehydrolimonoate A-ring lactone and 17-dehydronomilinoate A-ring lactone, respectively. Immobilized cells of *A. globiformis* II, on the other hand, converted limonin and nomilin to nonbitter limonol and nomilol, repectively.

We have recently isolated from soil another bacterium, *Corynebacterium fascians*, that is capable of metabolizing limonoids (Hasegawa and King, 1983). Among the organisms isolated, *C. fascians* is the only one that produces constitutive enzymes for the metabolism of limonoids. The others all require the presence of a limonoid inducer in their growth media to produce cells capable of metabolizing limonoids. The objective of this study was to reduce bitter limonin and nomilin in citrus juice sera to below bitterness thresholds with *C. fascians* grown on various carbon sources, immobilized in acrylamide gel and used in the biological process previously developed.

MATERIALS & METHODS

CITRUS FRUIT were purchased from a local market and juices were prepared with a Sunkist juicer. Linomin and nomilin were dis-

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solved in a minimal portion of acetronitrile and slowly stirred into the juices (5 – 7 ppm limonin and 1 – 2 ppm nomilin) to obtain convenient working concentrations. Juice sera were prepared from the juices by centrifugation at 4,000 x *g* for 20 min and kept in a freezer until used. Silica gel plates were used for analysis of limonoids and the plates were developed with cyclohexane-EtOAc (2:3), CH_2Cl_2-MeOH (97:3) or toluene-EtOH-H_2O-HOAc (200:47:15:1, upper layer).

Bacterial cells

Corynebacterium fascians was grown on mineral salt-0.2% nutrient broth media containing 0.4% carbohydrate of interest by the procedure described previously (Hasegawa and King, 1983). Cells were collected by centrifugation at 5,000 x *g*, washed with 0.5M potassium phosphate buffer at pH 7.0 and kept in a freezer until used.

Immobilization of cells

Cells were immobilized in acrylamide gel by the procedure of Tosa et al. (1974). The resulting gel was blended gently with a Polytron and packed in a 2-cm diameter column.

Treatments of juice sera

A 50 mL portion of juice serum was passed through the column at room temperature at a rate of 100 mL per hr. The column was washed with H_2O followed by 0.05M potassium phosphate at pH 7.0. The eluate and washings were pooled and used for limonoid analysis. The washed column was kept in a 5°C room until next use. For stability studies, limonin- or nomilin-containing juice serum (50 mL) was passed through a column containing 4g immobilized *C. fascians* cells. Column conditions and handlings of the column after each use were similar to those described above.

Analysis of limonoids

Combined column fractions of treated juice sera were acidified to pH 2 with 1N HCl and extracted with CH_2Cl_2 twice. The CH_2Cl_2 fractions were combined and evaporated to dryness and the residue was analyzed for limonoids by the procedure of Maier and Grant (1970).

Analysis of acids and sugars

The organic acids and sugars were measured by HPLC on a Bio-Rad HPX-87 column (7.8 x 300 mm) at 34°C. The mobile phase was 5 x 10^{-4}N H_2SO_4 at a flow rate of 0.6 mL per min. The acids were detected spectrophotometrically at 210 nm and the sugars were detected by refractive index. A Perkin-Elmer HPLC system (Series 4 Liquid Chromatograph) and detectors, Spectra-Physics (SP8400 UV/vis detector) and Micrometrics (771 refractive index detectors) were used.

RESULTS & DISCUSSION

CORYNEBACTERIUM fascians, isolated from soil by enrichment with 3-furoic acid as a single carbon source, produces constitutive enzymes involved in the metabolism of limonoids (Hasegawa and King, 1983). Cells were immobilized in acrylamide gel and used for the biological process we developed previously for the reduction of limonoid bitterness of citrus juice sera. Cells grown on fructose reduced limonin and nomilin contents of juice sera of navel orange, Valencia orange and grapefruit (Table 1). In each case, limonin and nomilin contents were reduced to below the bitterness thresholds (6 ppm each). These results confirmed

the presence of limonoid metabolizing enzymes in cells grown on fructose. Similar results were obtained with cells grown on galactose or citric acid.

A stability study of immobilized cells of *C. fascians* was conducted with navel orange juice sera (Table 2). Four gm of cells grown on fructose reduced the limonin content of the sera significantly in 10 different runs. The enzyme system in the immobilized cells was, however, less effective and less stable than that of *A. globiformis* (Hasegawa et al., 1982). Under similar conditions, immobilized cells of *A. globiformis* reduced the limonin content below 6 ppm (limonin bitterness threshold) and the same column was used 15 – 20 times without losing its effectiveness. Immobilized *A. globiformis* cells converted limonin to 17-dehydrolimonoate A-ring lactone which is catalyzed by limonoate dehydrogenase, one of the major limonoid-metabolizing enzymes present in bacteria. The immobilized cells of *C. fascians*, on the other hand, converted limonin to limonol which is catalyzed by limonol dehydrogenase, one of the minor limonoid-metabolizing enzymes present in bacteria (Hasegawa et al., 1983a). It is not clear why the immobilized cells of *C. fascians* converted limonin to limonol instead of 17-dehydrolimonoate A-ring lactone. This is apparently the main reason why the process with *C. fascians* cells was less effective and less stable than that of *A. globiformis* cells in limonin debittering.

Bitter nomilin of citrus juice sera was very effectively converted to a nonbitter compound by immobilized cells of *C. fascians*. Table 3 shows the results of a typical nomilin debittering test using grapefruit juice sera. The process reduced the nomilin content of the sera to almost 0 up to the 14th run. Therefore, data are presented in an abbrevi-

ated version. Even at the 20th run, the process reduced juice serum containing 20 ppm nomilin to 3.0 ppm. This represents an 85% reduction in nomilin content.

Unlike immobilized cells of *A. globiformis* and *A. globiformis* II, which convert nomilin to 17-dehydronomilinoate A-ring lactone and nomilol, respectively, immobilized cells of *C. fascians* convert nomilin to obacunone (Fig. 1; Hasegawa et al., 1984). The enzyme responsible for this conversion, nomilin acetyl-lyase, has been isolated from cell-free extracts of *C. fascians* (Herman et al., 1984). The organism contains very high activity of the enzyme: one gram of cells grown on limonoate, for example, possesses activity which catalyzes the conversion of approximately 10g of nomilin per min under the *in vitro* assay conditions used. This clearly explains why this organism very effectively converts nomilin to obacuonone in citrus juice sera and is an ideal one for nomilin debittering of citrus juices. The major bitter component in citrus juices, limonin, was, however, removed less effectively.

The effect of debittering treatments on the composition of navel orange juice sera is shown in Table 4. Analysis of variance showed that the concentration of citric, malic and ascorbic acids did not significantly change as a result of

Table 1—*Reduction of limonin and nomilin content of various citrus juice sera with* Corynebacterium fascians *cells immobilized in acrylamide gel*[a]

	Limonin content		Nomilin content	
Juice sera	Control (ppm)	Treated (ppm)	Control (ppm)	Treated (ppm)
Navel orange	20.0	3.0	21.5	0
	23.0	4.0	20.5	0
Valencia orange	20.0	3.0	19.5	0
	23.0	3.0	21.0	0
Grapefruit	21.5	4.5	22.0	0
	19.5	4.0	21.5	0

[a] A 50-mL portion of juice sera was treated with 5g cells grown on fructose. Details are given in the text.

Table 2—*Reduction of limonin content of navel orange juice serum by immobilized cells of* Corynebacterium fascians *grown on fructose*[a]

Run	Time after immobilization (days)	Limonin content		
		Control (ppm)	Treated (ppm)	Reduction (%)
1	1	21	4.0	81
2	3	21	4.5	78
3	5	21	4.8	77
4	8	23	5.1	79
5	10	23	6.0	74
6	12	23	7.2	69
7	15	21	7.8	63
8	18	21	6.8	68
9	21	21	8.8	58
10	23	23	10.5	54

[a] 50 mL of the serum was passed through a 2.0-cm diameter column packed with 4.0g cells immobilized in acrylamide gel.

Fig. 1—*Metabolism of limonin and nomilin in immobilized cells of* Corynebacterium fascians.

Table 3—*Reduction of nomilin content of grapefruit juice sera by* Corynebacterium fascians *immobilized in acrylamide gel*[a]

Run	Time after immobilization (days)	Nomilin content		
		Control (ppm)	Treated (ppm)	Reduction (%)
1	1	21.5	0	100
5	10	22.0	0	100
10	23	23.5	0	100
15	33	21.0	1.5	93
20	46	20.0	3.0	85
24	55	23.0	6.0	74

[a] 50-mL portion of grapefruit juice serum was passed through a 2.0-cm diameter column packed with 4g immobilized cells.

Table 4—Orange juice serum composition as affected by debittering treatment with Corynebacterium fascians grown on various nutrient media

| Samples | Bacterial growth media | Concentration (g/100 mL) | | | | | | (ppm) |
		Citric	Malic	Ascorbic	Sucrose	Glucose	Fructose	Limonin
1	Control[c]	1.58	0.10	0.043	2.17[a] (47%)	1.14 (24%)	1.36 (29%)	19.1
	Fructose	1.60	0.10	0.043	2.47[b] (46%)	1.31 (24%)	1.61 (30%)	7.5
	Galactose	1.52	0.10	0.036	2.66[b] (47%)	1.43 (25%)	1.61 (28%)	6.1
	Limonoate	1.59	0.11	0.041	2.96[b] (47%)	1.49 (24%)	1.68 (29%)	6.2
2	Control[c]	1.69	0.14	0.053	3.20[a] (48%)	1.60 (24%)	1.88 (28%)	20.0
	Fructose	1.73	0.13	0.053	3.47[b] (48%)	1.71 (24%)	2.02 (28%)	8.0
	Galactose	1.67	0.11	0.050	3.31[b] (48%)	1.64 (24%)	1.96 (28%)	7.2
	Limonoate	1.77	0.13	0.052	3.58[b] (49%)	1.71 (23%)	2.02 (28%)	7.5
3	Control[c]	1.56	0.11	0.054	3.31[a] (50%)	1.49 (23%)	1.74 (27%)	21.0
	Fructose	1.65	0.13	0.059	3.55[b] (51%)	1.60 (23%)	1.86 (26%)	8.1
	Galactose	1.68	0.13	0.060	3.69[b] (51%)	1.64 (23%)	1.92 (26%)	7.5
	Limonoate	1.64	0.11	0.057	3.71[b] (51%)	1.66 (23%)	1.92 (26%)	5.5
		Analysis of variance						
	Treatment	N.S.	N.S.	N.S.	0.05 (N.S.)	0.05 (N.S.)	0.05 (N.S.)	

[a,b]Means without the same superscript are significantly different ($P < 0.05$). No superscripts indicate no significant differences ($P > 0.05$).
[c] Control: treated with a column packed with acrylamide gel without bacterial cells.

the treatments. The concentrations of sugars, however, were slightly but significantly lower ($P < 0.05$) for the control than for the cell treated samples. The percentages of sugar composition shown in parenthesis, however, did not change. This is most likely due to adsorption of sugars on the acrylamide gel because the control column contained more gel than the immobilized cell column although total volumes of all columns were the same. Hence, it can be concluded that the use of *C. fascians* for debittering will not adversely affect the quality of juice.

The utilization of *C. fascians* for the biological process of limonoid-debittering of citrus juice is advantageous from a practical viewpoint. This is because cells which possess limonoid-metabolizing enzymes can be prepared conveniently and relatively cheaply by using inexpensive carbon sources. This contrasts with the other organisms which require a limonoid inducer in media. Presently citrus limonoids are not commercially available.

REFERENCES

Hasegawa, S. and Bennett, R.D. 1983. Metabolism of limonate via a trans-19-hydroxyobacunoate pathway. J. Agric. Food Chem. 31: 805.

Hasegawa, S., Bennett, R.D., Maier, V.P., and King, A.D. Jr. 1972. Limonoate dehydrogenase from Arthrobacter globiformis. J. Agric. Food Chem. 20: 1031.

Hasegawa, S., Choi, G., Herman, Z., and Ou, P. 1983a. Abstract. Isolation and utilization of Corynebacterium fascians for limonoid debittering of citrus juices. Citrus Research Conf. Pasadena, CA, p. 2.

Hasegawa, S., Dillberger, A., and Choi, G. 1984. Metabolism of limonoids: Conversion of nomilin to obacunone in Corynebacterium fascians. J. Agric. Food Chem. 32: 457.

Hasegawa, S. and Kim, K.S. 1975. Abstract. Biochemistry of limonoids. A new limonoid-debittering enzyme. Citrus Research Conf; Pasadena, CA, p. 15.

Hasegawa, S. and King, A.D. 1983. A species of bacterium producing constitutive enzymes for limonoid metabolism. J. Agric. Food Chem. 31: 807.

Hasegawa, S., Maier, V.P., Border, S.N., and Bennett, R.D. 1974b. Metabolism of limonoids: Isolation and characterization of deoxylimonin hydrolase from Pseudomonas. J. Agric. Food Chem. 22: 1093.

Hasegawa, S., Maier, V.P., and King, A.D. Jr. 1974a. Isolation of a new limonoate dehydrogenase from Pseudomonas. J. Agric. Food Chem. 22: 523.

Hasegawa, S. and Pelton, V.A. 1983. Reduction of nomilin bitterness in citrus juices and juice serum with Arthrobacter globiformis cells immobilized in acrylamide gel. J. Agric. Food Chem. 31: 178.

Hasegawa, S., Pelton, V.A., and Bennett, R.D. 1983b. Metabolism of limonoids by Arthrobacter globiformis II: Basis for a practical means of reducing the limonin content of orange juices by immobilized cells. J. Agric. Food Chem. 31: 1002.

Hasegawa, S., Patel, M.N., and Snyder, R.C. 1982. Reduction of limonin bitterness in navel orange juice serum with bacterial cells immobilized in acrylamide gel. J. Agric. Food Chem. 30: 509.

Herman, Z., Hasegawa, S., and Ou, P. 1985. Nomilin acetyl-lyase, bacterial enzyme for nomilin debittering of citrus juices. J. Food Sci. 50: 118.

Maier, V.P. and Grant, E.R. 1970. Specific thin-layer chromatography assay of limonin, a citrus bitter principle. J. Agric. Food Chem. 18: 250.

Tosa, T., Sato, T., Mori, T., and Chibata, I. 1974. Basic studies for continuous production of L-aspartic acid by immobilized Escherichia coli cells. Appl. Microbiology 27: 886.

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Effects of pH, Storage Time and Temperature on the Tin Content of Single Strength Canned Grapefruit Juice

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----------ABSTRACT----------

Grapefruit juice modified to pH 3.0, 3.5 and 4.0 and stored at 4, 21, 27, 32 and 38°C manifested a consistent two-stage pattern for tin uptake. The first stage consisted of a rapid (3 wk) uptake of tin (from 12 to approximately 100 ppm) and was relatively independent of juice pH or storage temperature. In the second stage, the increase in tin concentrations was extremely temperature dependent and only slightly pH dependent. Canned grapefruit juice should be stored below 27°C to insure tin contents do not exceed the Codex maximum of 250 ppm. In a survey of 55 commercially canned grapefruit juice samples stored 1 – 8 months under commercial conditions, tin concentrations ranged from 34 – 180 ppm.

INTRODUCTION

THE BULK of the grapefruit juice produced in the United States is sold as single-strength grapefruit juice packed in unlined, tin-coated steel cans. Since grapefruit juice has both acidic and complexing properties, it will attack the tin layer during storage. The rate of tin uptake by grapefruit juice is dependent upon a number of factors. Tin concentrations up to 250 ppm are acceptable (Codex Alimentarius Commission, 1978) and have come to be regarded as the maximum limit for foods stored in unlaquered cans (Debost and Cheftel, 1979). Toxicity studies have shown that tin does not accumulate in organ tissues of rabbits or rats (Kutzner and Brod, 1971; Fritsch et al., 1977). Tin is relatively nontoxic because most ingested tin is not absorbed during the digestion process and is excreted in the feces (Calloway and McMullen, 1966; Hiles, 1974). However, Benoy et al. (1971) reported gastrointestinal disturbances in cats and human volunteers following the drinking of fruit juice prepared to contain at least 1400 ppm of tin.

Increased tin content of canned citrus juices is an undesirable but apparently necessary result of storage in tin-plated steel. It is undesirable because elevated tin levels are associated with the development of a metallic off-flavor in the juice. However, increased tin concentrations are a necessary trade-off so that loss of vitamin potency and browning may be minimized during storage. Attempts to reduce the tin content through the use of lacquered cans produced juices with significant discoloration (Mannheim and Hoenig, 1971). Apparently, tin acts as a sacrificial anode, being preferentially oxidized instead of other labile juice components such as carotenoids and vitamin C.

The corrosion process is dependent on a number of physical and chemical factors. Bakal and Mannheim (1966) studied the effects of processing variables such as deaeration, improper cooling and increased headspace on the rate of can corrosion and juice quality. Added chemical parameters such as the role of sulfur dioxide and nitrate on detinning of canned graepfruit juice have been studied by Saguy et al. (1973). Nagy et al. (1980) studied the effects of storage temperature and time on the iron and tin content of commercial canned single strength orange juice. Bird

(1980) developed an equation to estimate tin uptake of citrus products stored in unlacquered cans based on the alloy-tin couple, tin crystal size, and porosity. Unfortunately, the equation assumes uniform detinning, a factor which at present may not be controlled.

The purpose of this study was to quantify how different storage conditions (time-temperature) and juice pH contribute to the accumulation of tin in canned grapefruit juice and determine which are the dominant factors so that recommendations could be made to minimize tin uptake during storage. Since commercial juices are prepared and stored in a slightly different manner, a survey of grapefruit juice from several Florida processors was undertaken to determine tin levels in commercial product.

MATERIALS & METHODS

Instrumentation

A Perkin-Elmer (P-E) model 503 atomic absorption spectrophotometer (AAS) was used with a P-E tin electrodeless discharge lamp (EDL) set at 8 watts. Tin absorbance was measured at 224.6 nm with a slit width of 0.2 nm. A single slot nitrous oxide-acetylene burner head was used. The nitrous oxide-acetylene gas flame was adjusted to give a fuel rich (reducing) flame. Burner head height and horizontal alignment were adjusted to give maximum absorption with a tin standard. Nebulizer aspiration rate was adjusted to produce the manufacturer's specified absorbance with a 200 ppm Sn solution.

The atomic absorption spectrophotometer was run in the concentration model using a 50 ppm Sn solution to calibrate the instrument. A blank containing the same acids at identical concentrations as the samples was used to zero the instrument. Three concentration readings taken during 1 sec integrations were recorded and averaged for each sample.

Samples were digested on a Technicon model BD 20/40 sample digestor.

Reagents

The tin was prepared from AR grade tin metal, dissolved in hydrochloric acid (HCl) and diluted with distilled deionized water to make the final solution 10% HCl (v/v). Working standards were prepared by diluting the tin standard using 10% HCl. All mineral acids used were of reagent grade.

Canned juice preparation and storage

Juice was extracted from Florida Duncan grapefruit (*Citrus paradisi*) using an FMC Model 591 extractor and divided into three 75-liter lots. The initial pH of the juice was 3.2. Two of the lots were adjusted with citric acid and potassium hydroxide to pH 3.0 and 4.0, respectively. These would represent the extremes of acidity likely to be encountered in citrus other than lemon and lime. The last lot was adjusted to an intermediate acidity (i.e. pH 3.5) using potassium hydroxide.

All juices were pasteurized at 90.6°C and hot-filled (approx 82°C) into 211 × 304 (256 mL or 8 oz) cans (Can Division, Crown Cork and Seal Co., Bartow, FL). The can body interior and one end was 1.00 lb tinplate with a soldered side seam. The can factory end was enamel coated inside. Each can was hand sealed using a Dixie Canner Equipment Co. (Athens, GA) sealer Model SD-1P-23. After sealing, the cans were cooled with a water spray and allowed to reach ambient temperature (approx 22°C) and stored at various temperatures with the enamel end up. The cans from each lots were distributed among five constant temperature rooms set at 4, 21, 27,

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32, or 38°C. Two cans of each lot from 4, 21 and 27°C were analyzed every 3 – 5 wk for 1 yr. Two cans from each lot stored at 32 and 39°C were analyzed at the same frequency but for only 6 months. The high temperature studies were terminated after 6 months because longer storage at these temperatures would be extremely unlikely in the industry.

Once removed from their storage rooms, the cans were allowed at least 1 hr to equilibrate to room temperature. Each can was thoroughly shaken before opening. Total juice volume was measured and recorded. Head space was determined from the difference between juice volume and the volume of the can.

Sample preparation

Five mL of single strength juice was pipetted into a 22 x 200 mm acid-washed test tube. To each tube was added: 3 mL of conc HNO_3, 1 mL conc $HClO_4$ and 1 mL of conc. H_2SO_4. The contents of the tubes were mixed by swirling gently. Digestion was accomplished by heating the juice-acid mixture in heated aluminum blocks at 80°C for 3 hr then at 200°C for 2 hr. The resulting clear, colorless solution was cooled and diluted to 25 mL with 10% HCl.

RESULTS & DISCUSSION

Sample preparation

Price and Ross (1969) reported analyzing tin and iron using atomic absorption spectroscopy by simply diluting and filtering orange juice. This approach was highly attractive because of its speed and simplicity. However, preliminary experiments indicated that pulp and insoluble pectin from canned grapefruit juice contained tin. Therefore, the entire sample was digested to eliminate the possibility of low tin results. Dry ashing of grapefruit juice samples was found to be too time consuming to be used for a large number of samples and there were problems with incomplete ashing after heating up to 500°C for 12 hr. Therefore, juice samples were digested using a mixture of nitric, perchloric and sulfuric acids and a two-step heating procedure. The acidified juices were initially heated at 80°C as sample loss occurred from foaming because the reaction was too vigorous at temperatures greater than this. The final heating

step (200°C) effectively removed the nitric acid and at the same time increased the oxidative power of perchloric acid.

Capacho-Delgado and Manning (1966) studied the effects of various mineral acids on tin absorption in air-hydrogen and air-acetylene flames. Air-hydrogen had the greatest sensitivity but was susceptible to interference from several mineral acids. Certain mineral ions (Juliano and Harrison, 1970) also interfered. The nitrous oxide-acetylene flame was chosen for this study because it is relatively free from interferences and because expected tin concentrations would be high enough that extreme sensitivty would not be needed.

To determine if the combination of mineral acids affected the tin absorbance, known amounts of standard tin solutions were added to the diluted ashed juice samples and their respective absorbances measured. As illustrated in Fig. 1, the slope of the resulting standard addition is identical to the slope from the tin standards. This indicates that there is no suppression or enhancement of the tin absorbance due to the digesting acids and tin concentrations can be calculated using the method of external standards.

Storage time

Initial tin concentration of freshly processed juice was 12 ppm. After 3 wk of storage, tin concentrations of the same juice increased to 80 – 140 ppm. Subsequently, tin concentrations stabilized or increased dramatically depending on storage temperature and pH. This general behavior is illustrated in Fig. 2 for pH 3.0 juices stored at 38 and 4°C. The rapid uptake of tin within the first 3 wk followed by the more gradual temperature dependent uptake during the remaining 48 wk of the study suggest the possibility of two separate mechanisms for tin uptake by grapefruit juice. Since oxygen was not excluded from the head space gases, nor were the juices deaerated, the rapid uptake of tin during the first 3 wk storage is probably associated with an oxygen reaction. Kefford et al. (1959) measured oxygen levels in canned orange juice and found that it had completely reacted within the first 100 hr of storage.
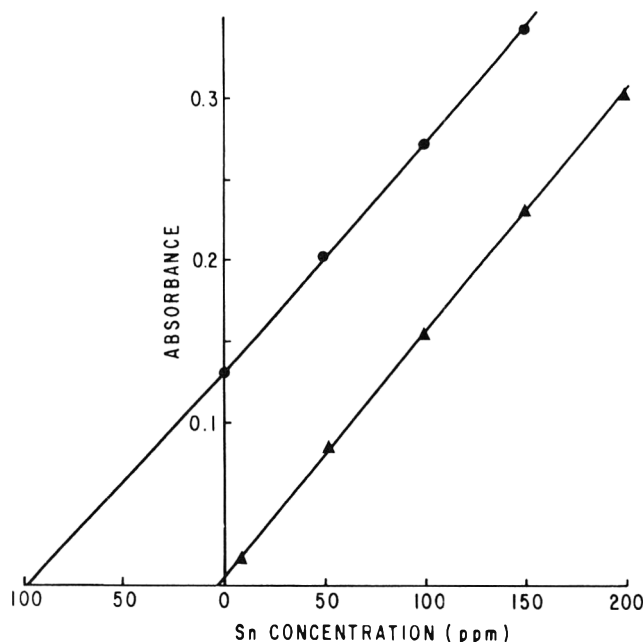
Fig. 1—Standard additions of tin to an acid digested grapefruit juice sample: ● = juice sample; ▲ = calibration plot with standard tin solutions.
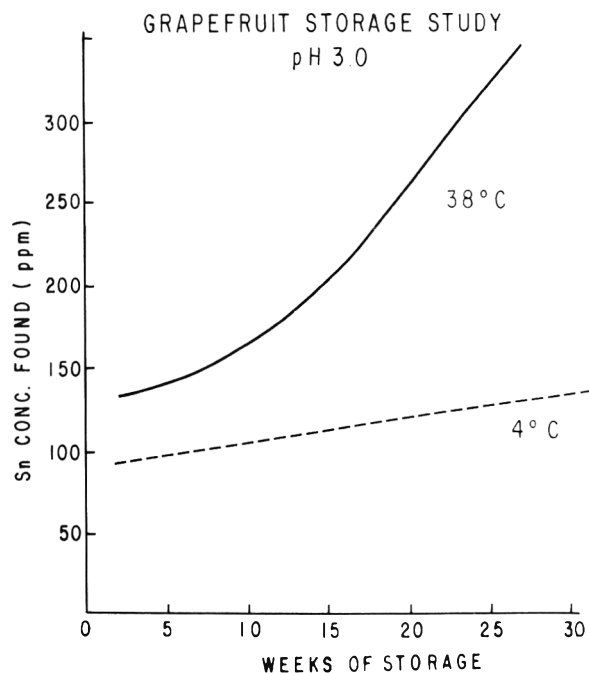
Fig. 2—Effect of storage temperature on tin concentrations of grapefruit juice stored for 6 months.

Temperature

The major effect of storage temperature was not the initial tin uptake, but the subsequent rate at which grapefruit juice tin concentration increased with time. As shown in Fig. 3, storage temperature had little effect on juice tin concentrations during the first 3 wk of storage. Subsequently, the effect of storage temperature became pronounced (Fig. 4). After 3 wk storage at 4°C, pH 3.0 grapefruit juice had a tin content of approximately 120 ppm. Subsequently, tin content increased slowly with storage time and the rate of increase was linear. Juice stored at 38°C had only a slightly greater tin content (approximately 140 ppm) after 3 wk storage. However, the subsequent rate of tin uptake for juice stored at 38°C was considerable, increasing at a rate of 12 ppm tin per week after 10 wk storage. The rate of tin uptake was also pH-dependent because the rate of tin uptake for the pH 3.5 and 4.0 juices were both 8 ppm per week. Thus if tin concentrations are to be minimized, canned grapefruit juice should be stored at as low a storage temperature as possible and certainly below 27°C. Lower storage temperatures will not only reduce tin content but will also result in a juice with better flavor and higher vitamin C content (Bakal and Mannheim 1966).

Head space

At every pH and storage temperature, there was a rapid increase in tin concentration from the initial 12 ppm to approximately 100 ppm after 3 wk storage, regardless of juice pH or storage temperature. Such behavior suggests that oxygen dissolved in the juice or entrapped within the can headspace reacted rapidly with tin metal to form stannous ions. After dissolved and headspace oxygen is depleted, an anaerobic detinning mechanism, which is primarily temperature dependent, takes over.

Since the cans were hand filled, can headspace was difficult to control. Juices from the cans with greater headspace contained greater amounts of tin. Of all the headspace gases, oxygen is the one most likely to react with tin (metal) because of the favorable electrochemical potential between these two elements. Bakal and Mannheim (1966) and Saguy et al. (1973) also reported greater tin concentrations in canned grapefruit juices with larger head-

spaces. Nishijuma et al. (1971) reported finding two to four times greater tin concentrations in acidic fruit juices allowed to stand 72 hr in the opened can. Royo-Iranzo et al. (1970) reported reducing detinning of canned orange juice packed with a 45 – 50 cm Hg vacuum. Thus it appears that can headspace plays an important part in the rapid detinning of canned grapefruit juice.

Acidity (pH)

An inverse relationship between pH and juice tin concentrations was observed at all storage temperatures. The influence of hydrogen ion was not unexpected because it is generally recognized that hydrogen gas is formed during the detinning process (Dickinson, 1961). Thus the greater the hydrogen ion concentration (lower the pH) the more readily it reacts with metallic tin to form hydrogen gas and ionic tin. The tin content of less acidic juices increased much more slowly with increasing storage time. Below 32°C the rate of tin increase of the pH 4.0 juices was about one-half that of the pH 3.0 juice.

Commercial survey

Fifty-five samples of Florida grapefruit juice packed in 46 oz. cans were analyzed for tin. As seen in Table 1, the average pH of the juices remained constant. Average and median tin concentrations increased with storage time. It is interesting to note that even after more than 2 months of storage (January pack) the average and median tin concentrations were less than those observed under experimental conditions after only 3-wk storage. The most likely explanation is that the commercial juices were deaerated to remove as much oxygen as possible before canning, whereas the experimental juices were not. Can sizes were also different and the relative amount of headspace to juice volume was also smaller in commercial juice. Thus, in commercial juices the amount of oxygen that could react with tin was smaller than in the test pack and the resulting tin values were smaller.
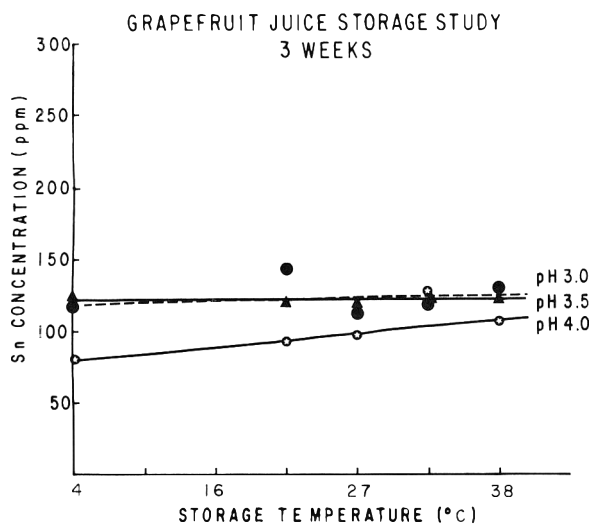
Fig. 3—Effect of storage temperature on pH on tin concentrations of grapefruit juice stored for 3 wk. • = pH 3.0; ▲ = pH 3.5; ○ = pH 4.0.
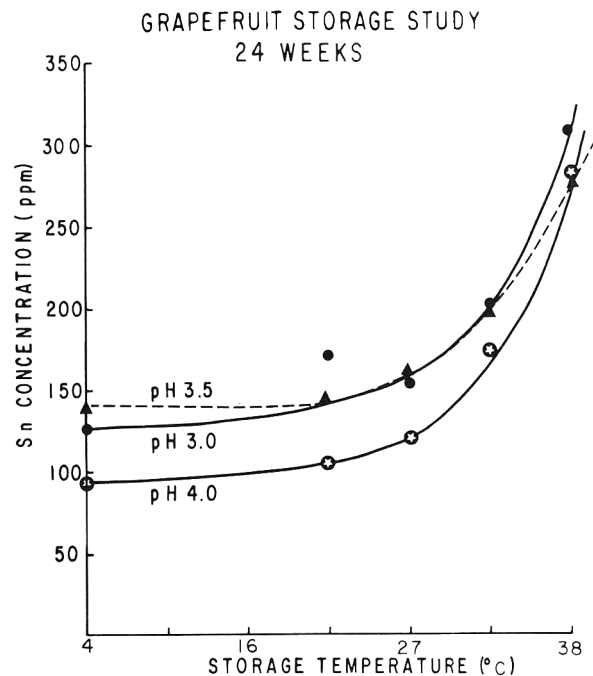
Fig. 4—Effect of storage temperature and pH on tin concentrations of grapefruit juice stored for 24 wk.

Aciduric and Heat Resistant Microorganisms in Apple Juice and Cider Processing Operations

K. M. J. SWANSON, S. B. LEASOR, and D. L. DOWNING

ABSTRACT

Site visits to 15 apple juice or cider facilities were conducted in New York State. Washed apples, pulp, press juice, final product, and other samples, when appropriate, were sampled aseptically. Samples were plated on acidified potato dextrose agar, before and after heating at 70°C for 1 – 2 hr. For unheated samples, molds predominated in fruit, yeasts and molds in pulp, and yeasts in press and final juice samples. Average counts were 2.8×10^4, 7.3×10^4, 1.7×10^5, and $1.4 \times 10^5/g$, respectively. Heat resistant yeasts, molds, and bacteria were isolated frequently, generally at a level of less than 1 per 10g.

INTRODUCTION

APPLE JUICE is a product which is currently growing in popularity due to interest in "healthy" foods and, perhaps, to increased use of aseptic packaging. National trends indicate that while the consumption of other fruit juices is declining, apple juice consumption is increasing and will probably continue to do so (Anon., 1983). Despite the increased demand for apple juice in the marketplace, little work is being conducted on the natural microbial flora of the product. Marshall and Walkley (1951) and Beech (1958) conducted quantitative and qualitative work in this area. With recent advances in processing technology, reevaluation of the flora seems warranted, particularly in regard to the presence of heat resistant aciduric microorganisms in the product.

Put et al. (1976) have described heat resistant yeasts isolated from soft drinks. Heat resistant molds were isolated frequently in orchards (Splittstoesser et al., 1971), and studies have been conducted on thermal resistance of spoilage organisms in orange juice (Juven et al., 1978). Knowledge of the prevalence of heat resistant aciduric microorganisms in apple juice, however, is lacking.

Therefore, the objectives of this work were to characterize the aciduric microflora of apple juice and sweet cider during processing, and to determine the prevalence of heat resistant aciduric microbes in these operations.

MATERIALS & METHODS

Site visits

Site visits to 15 apple juice or sweet cider facilities were conducted in New York State during the 1983 harvest season. Plant sizes ranged from seasonal operations producing approximately 2000 gal/day to year round facilities capable of producing 3000 gal/hr. Operators were queried on cleanup and sanitation procedures as well as expected product shelf life.

Daily variation in the aciduric population of press juice was monitored in one hydraulic press plant. In this plant, press cloths were washed and dried, and equipment sanitized at the end of each day's production. On each of six days, juice was sampled from the

first run in the morning, the first run following the noon break, and the last run of the day. Total aciduric counts were determined as described below.

Microbiological analyses

At each operation, one sample each of washed apples, pulp, juice from the press, and final product were obtained aseptically. When appropriate, samples of juice before and after filtration, pulp with and without press aid, and final product with and without preservatives were also obtained. Preservative concentrations were not measured. Samples were held on ice for <24 hr. Solid and semi-solid samples were diluted with an equal weight of sterile distilled water and blended prior to further dilution (0.1% peptone water) and plating. Samples were plated, in duplicate, on acidified potato dextrose agar (pH 3.5, PDA) and incubated for 5 days at 25°C prior to enumeration of colonies. A gross estimate of the mold: yeast ratio was recorded. Microscopic evaluation of selected "yeast-like" colonies was employed to distinguish yeast from bacteria.

A modification of the method of Splittstoesser (1976) was used to isolate heat resistant aciduric microorganisms from samples. One hundred milliliters juice or 200g diluted and blended solids were held in a 70°C water bath for 1 or 2 hr, respectively, including come up time. Following heating, 10 mL were delivered to each of four petri dishes, and an equal volume of acidified PDA was added. Plates and the remainder of the heated sample were incubated at 30°C for 4 wk. Morphological characteristics of any growth was described, and isolates were streaked on PDA slants. To verify heat resistance of isolates, 5 mL apple juice in 13 x 100 mm screw-capped tubes, were inoculated with growth from the slant using a wire loop. No attempt was made to examine cultures for potential ascospore production prior to the verification test, and population levels were not standardized. Juice was heated at 70°C for 15 and 30 min in a circulating water bath with the water level close to the top of the tube. Heated juice was incubated at 30°C for 3 wk and turbidity was compared to both uninoculated and unheated controls.

Yeasts were identified using the methods of Lodder (1971), in conjunction with API 20C yeast identification strips. Methods described by Arx (1970) were used for *Aureobasidium* classification. Bergey's manual (Buchanan and Gibbons, 1974) and Gordon et al. (1973) were used for bacterial identification. Identification of molds was done by macroscopic and microscopic examination of cultures on mycological agar by D. Prest (Keuka College, Keuka Park, NY).

Statistical analysis

The significance of differences between consecutive samples was determined using a Student's *t*-test for paired differences (Snedecor and Cochran, 1967). Data were transformed to a logarithmic scale for normalization prior to statistical calculations.

RESULTS & DISCUSSION

TABLE 1 lists total aciduric populations at various stages of apple juice processing for 15 operations. There were no apparent relationships between operation size and populations of aciduric microorganisms during processing. The aciduric population of fruit ranged from 3.4×10^3 to $4.7 \times 10^5/g$ (Table 1), and >60% of these microorganisms were molds (Fig. 1). The remaining organisms were generally yeasts, with the exception of one plant in which aciduric bacteria predominated in fruit (Plant No. 3, Table 1). Two peel and core samples had total aciduric populations approximately ten-fold greater than the geometric mean population of solid fruit (Table 1).

The aciduric population of ground pulp was significantly greater (p < 0.05) than that of apples (Fig. 2). The increase may have been due to contaminated equipment and/or sample uniformity. While press aids had similar or lower aciduric populations than pulp, the addition of press aid seemed to increase the aciduric population of pulp in individual operations (Table 1). This increase may be attributed to inadequate sanitation and/or to release of trapped microbes by the press aid. Approximately 60 – 70% of the microflora in pulp was yeast and 30 – 40% was mold (Fig. 1). Yeasts greatly predominated in pulp with press aid.

Marshall and Walkley (1951) reported that the microbial population of freshly expressed juice was less than that of pulp. In our studies, the total aciduric population of juice was generally significantly greater (p < 0.05) than that of pulp when press aids were not used (Fig. 2). The levels of aciduric microbes found were similar to those reported by Beech (1958). Yeasts predominated in press juice, and molds predominated in pomace (data not presented), suggesting that molds were trapped in the pomace during pressing.

Fig. 3 illustrates the changes in the aciduric population of press juice during the processing day. An overall trend of increasing counts was observed during daily production. The increase was probably due to microbial growth in press cloths and on racks (Marshall and Walkley, 1951; Beech, 1958). The daily increase was approximately one doubling, and was less than the day to day, minimum to maximum variation. Consequently, while midday cleanup would not be harmful, the beneficial effect of this procedure would be limited. Several plants did not employ daily cleaning of press cloths, and significant microbial buildup could occur in these facilities.

While a depectinization step was employed in only four of the plants surveyed, these facilities produced the largest volume of juice. The average aciduric population of depectinized juice was similar to that of press juice for other plants (Fig. 2). For a given operation, however, depectinization reduced the aciduric population of the juice (Table 1). Marshall and Walkley (1951) reported an average microbial population reduction of 68% during fining, suggesting that microorganisms present in the juice settled out with other particulate matter. The use of depectinization by smaller operations may therefore be beneficial in producing a product with a lower population and a subsequent longer shelf life.

Diatomaceous earth (DE) filtration or ultrafiltration not only clarified juice, but also reduced the population of aciduric microorganisms (Fig. 2). Population reductions of one to two log cycles were achieved by DE filtration alone. This reduction was far greater than the 3 – 4% reduction reported by Marshall and Walkley (1951). Advances in filtration technology have apparently increased the efficacy of the process.

The average aciduric population of unpasteurized juice was ca. 10^5/mL (Fig. 2). While no attempt was made to measure shelf life, discussions with operators indicated that the shelf life of unheated, refrigerated juice was generally 1 – 2 wk. Similar shelf lives were reported by processors who used sorbate in juice with high populations. This apparent lack of increased shelf life suggests that sorbate may have been used sometimes inappropriately as a substitute for proper sanitation and quality control, preventing the realization of maximum benefits. Further work in this area would be beneficial to the unpasteurized apple juice industry.
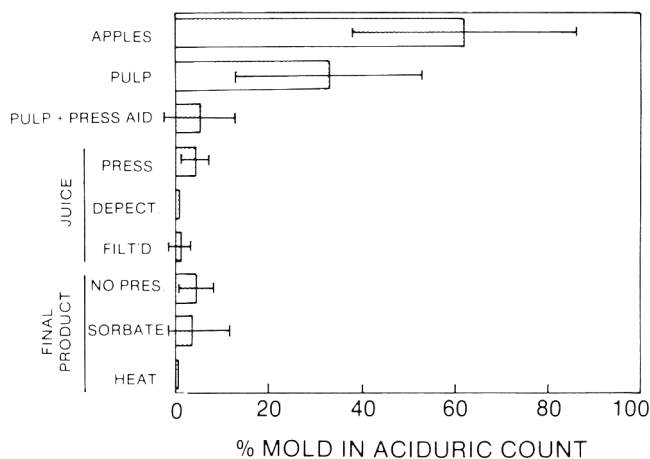
Fig. 1—Approximate percentage of molds present in the total aciduric population, enumerated on acidified potato dextrose agar (pH 3.5), during apple juice and cider processing in 15 plants. Bars (I) represent 95% confidence intervals. The remainder of the population was generally yeast.

Table 1—Aciduric microbial population during apple juice and cider processing at 15 facilities. Single samples of each category were taken at each location

| | | | | | | | Aciduric colony forming units (CFU) X 10^4/g | | | | | |
| | | | | | | | Juice | | | Final product | | |
	Capacity (gal)	Press	Apples	Pulp	Press aid	Pulp + press aid	Press	Depectinized	Filtered	No preservative	Added sorbate	Heated
1.	2,000/day	Hydraulic	47[a]	120	—	—	180	—	—	180[b]	—	—
2.	2,000/day	Hydraulic	1.4	0.98	—	—	4.3	—	—	4.3	—	—
3.	2,000/day	Hydraulic	NT	NT	—	—	NT	—	—	11	—	—
4.	30,000/yr	Hydraulic	1.9	75	—	—	38	—	—	33	—	—
5.	30,000/yr	Hydraulic	0.34	2.4	—	—	12	—	—	11	—	—
6.	500,000/yr	Hydraulic	1.4	1.3	—	—	4.2	—	—	5.4	6.7	—
7.	500,000/yr	Hydraulic	1.0	9.6	—	—	6.7	—	—	11	—	—
8.	750,000/yr	Hydraulic	18	47	—	—	20	—	—	—	42	—
9.	750,000/yr	Hydraulic	1.9	1.2	—	—	8.6	—	—	—	19	—
10.	750,000/yr	Jones	12[a]	NT	7.0R<0.01C	43	21	—	—	21[c]	—	—
11.	1,000,000/yr	Hydraulic	1.6	2.2	—	—	38	—	—	—	—	0.01 est.
12.	1,000,000/yr	Bucher	1.0	3.6	—	—	26	—	0.37[d]	—	—	0.00025
13.	2,000,000/yr	Ensink/Reitz	7.6	15	13R	290[e]	87	47	0.0066[f]	—	—	<0.0001[g]
14.	3,000,000/yr	Jones	NT	22	4.5R 0.22C	23	22	NT	0.14[d]	0.18	—	—
15.	3,500,000/yr	Jones	NT	12	NT	190	49	3.4	0.75[d]	—	—	<0.0001

[a] Peels and cores; [b] Used for vinegar production; [c] Processed further at another location; [d] DE filtration; [e] Second pressing; [f] Ultrafiltration; [g] concentrate; NT = not tested, R = rice hulls, C = cellulose, dash (—) = not used in process sampled.
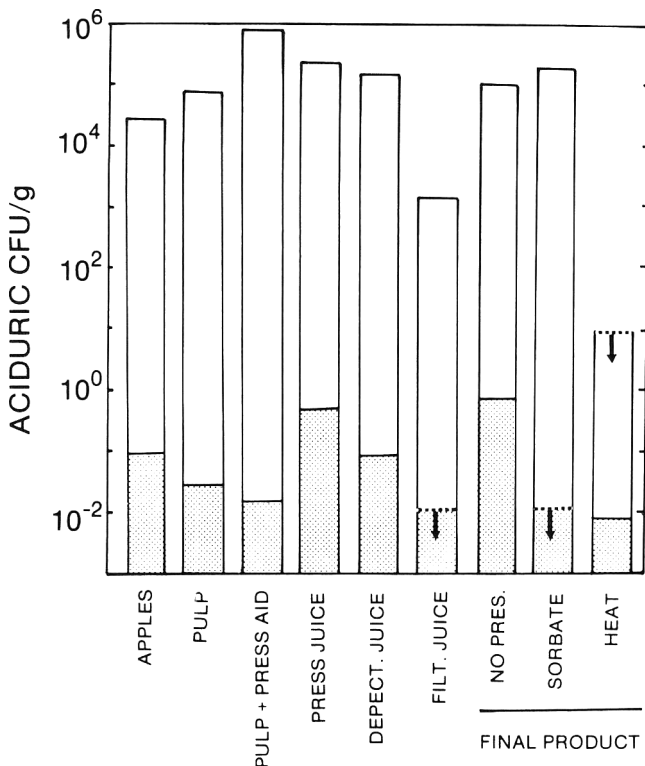
Fig. 2—Average total aciduric population (geometric means, □) and heat resistant aciduric population (arithmetic mean, □) during apple juice and cider processing in 15 plants. Arithmetic mean used for heat resistant population to facilitate inclusion of data for samples from which no heat resistant forms were recovered. Arrows indicate that the actual mean was less than the plotted value (i.e., no organisms were detected in some samples).

Table 2—Heat resistant microorganisms isolated from apple juice and cider processes

Group	No. of isolates
Bacteria	
Bacillus brevis	1
B. coagulans	2
Gluconobacter-like	3
Molds	
Aspergillus spp.	6
Byssochlamys/Paecilomyces	7
Penicillium spp.	5
Unidentified	3
Yeasts (Yeast-like)	
Aureobasidium	4
Candida krusei	1
Cryptococcus albidus	1
Kloeckera apiculata	1
Pichia vini	1
Rhodotorula rubra	1
Saccharomyces cerevisiae	2
Sacch. chevalieri	2
Sacch. rosei	1
Torulopsis glabrata	1
Unidentified	2

Heat treatments significantly reduced the microbial population of juice (Fig. 2). While no standard heat treatment was used, juice was generally heated to >90°C. Organisms were recovered from heated products that were subsequently refrigerated (Plants 11 and 12, Table 1), but not from shelf stable products (Plants 13 and 15, Table 1). Following heating, the refrigerated products were
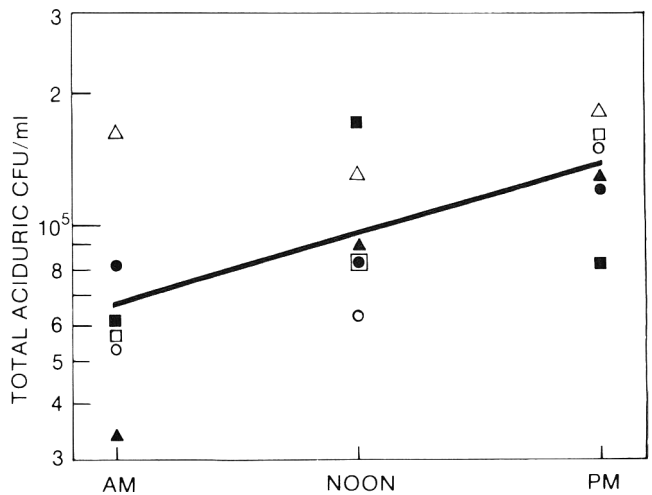
Fig. 3—Daily variation in total aciduric population, enumerated on acidified potato dextrose agar (pH 3.5), in apple juice from a hydraulic press in one operation. Line represents the best fit regression of all data; day 1, 2, 3, 4, 5 and 6 indicated by □, △, ●, ○, ■, and ▲, respectively.

cooled rapidly while shelf stable products were cooled slowly at ambient temperature. Apparently, the residual effect of heat during cooling contributed significantly to the total lethality of the process.

Heat resistant aciduric microorganisms were frequently isolated in apple processing operations at low levels (Fig. 2). It is probable that the selective heat treatment inactivated a portion of these organisms prior to isolation, making the initial population actually greater than that reported. A total of 99 isolates was found, 44 of which were verified to have some heat resistance. The fact that no attempt was made to promote formation of ascospores or other heat resistant structures prior to verification suggests that at least some of the unverified cultures may indeed be heat resistant. All verified cultures should be viewed as having some degree of heat resistance since a portion of the population survived at least two heat treatments at 70°C. However, it is not possible to evaluate the degree of heat resistance with existing data since the initial population is unknown.

Heat resistant microbes were found in all types of samples with the exception of filtered juice and juice with sorbate. While the limited availability of these samples may contribute to lack of recovery, sorbate has been shown to influence heat survival of acidurics (Beuchat, 1981). The population of heat resistant acidurics was generally less than 1 per 10g (Fig. 2), although populations as high as 5/g were recovered. In this instance, the isolate was an *Aureobasidium*, an organism which is not uncommon in apple juice (Seibt et al., 1984). Other isolates are listed in Table 2. The *Bacillus* spp. grew very slowly or not at all in apple juice. The *Gluconobacter*-like organisms were gram-negative coccobacilli, generally existing in paris and occasionally producing long cells or chains. Two isolates became gummy or mucoid with prolonged incubation. All of these isolates were capable of slow but definite growth in apple juice, and possessed unusual heat resistance for non sporulating species. Many of the asporogenous yeast isolates may be imperfect forms of sporogenous genera that have lost the ability to sporulate. Put et al. (1976, 1977) described a number of heat resistant yeasts isolated from soft drinks and fruit products. Heat resistance of *Byssochlamys* is well known (Splittstoesser et al., 1974), and *Aspergillus* and *Penicillium* spp. have also been reported to be heat resistant (Splittstoesser et al., 1971).

It is apparent that heat resistant aciduric microorganisms are present in apple juice processing environments. Thermal death time studies are necessary to properly evaluate the heat resistance of these microorganisms. Data generated will facilitate evaluation of heat treatments used in the processing of acidic foods. Sporodic spoilage episodes do occur in heat treated apple juice products and will continue if adequate thermal processes based on the kinetics of heat resistance of aciduric microorganisms are not developed.

REFERENCES

Anon. 1983. State of the food industry. Food Eng. 55: 67.

Arx, J.A. von. 1970. "The Genera of Fungi Sporulating in Pure Culture." Verlag von J. Cramer, Lehre, Germany.

Beech, F.W. 1958. The yeast flora of apple juices and ciders. J. Appl. Bacterial. 21: 257.

Beuchat, L.R. 1981. Synergistic effects of potassium sorbate and sodium benzoate on thermal inactivation of yeasts. J. Food Sci. 46: 771.

Buchanan, R.E. and Gibbons, N.E. (Ed.). 1974. "Bergey's Manual of Determinative Bacteriology," 8th ed. Williams and Wilkins Co., Baltimore, MD.

Gordon, R.E., Haynes, W.C., and Pang, C.H. 1973. The genus Bacillus. Agric. Handbook No. 427, U.S. Dept. of Agriculture, Washington, DC.

Juven, B.J., Kanner, J., and Weisslowicz, H. 1978. Influence of orange juice composition on the thermal resistance of spoilage yeasts. J. Food Sci. 43: 1074.

Lodder, J. (Ed.). 1971. "The Yeasts," 2nd ed. North-Holland Publishing Co., Amsterdam.

Marshall, C.R. and Walkley, V.T. 1951. Some aspects of microbiology applied to commercial apple juice production. 2. Microbiological control of processing. Food Res. 16: 515.

Put, H.M.C., de Jong, J., and Sand, F.E.M.J. 1977. The heat resistance of ascospores of Saccharomyces cerevisiae strain 195 and Saccharomyces chevalieri strain 215, isolated from heat preserved fruit juice. Spore Res. 1976. 2: 545.

Put, H.M.C., de Jong, J., Sand, F.E.M.J., and van Grinsven, A.M. 1976. Heat resistance studies on yeast spp. cuasing spoilage in soft drinks. J. Appl. Bacteriol. 40: 135.

Seibt, S.L., Weihe, J.L., and Hatcher, W.S. Jr. 1984. Growth and thermal resistance characteristics of a black pigmented yeast isolated from apple juice. Paper No. 230, presented at 44th Annual Meeting of Inst. of Food Technologists, Anaheim, CA, June 10 – 13.

Snedecor, G.W. and Cochran, W.G. 1967. "Statistical Methods," 6th ed. The Iowa State University Press, Ames, IA.

Splittstoesser, D.F. 1976. Enumeration of heat resistant mold (Byssochlamys). Ch. 17. In "Compendium of Methods for the Microbiological Examination of Foods," (Ed.) M.L. Speck, p. 230. American Public Health Association, Washington, DC.

Splittstoesser, D.F., Einset, A., Wilkison, M., and Preziose, J. 1974. Effect of food ingredients on the heat resistance of Byssochlamys fulva ascospores. Proc. IV Int. Congress Food Sci. and Technol. 3: 79.

Splittstoesser, D.F., Kuss, F.R., Harrison, W., and Prest, D.B. 1971. Incidence of heat-resistant molds in Eastern orchards and vineyards. Appl. Microbiol. 21: 335.

TIN CONTENT OF SS CANNED GRAPEFRUIT JUICE . . .

Table 1—Tin content of commercially canned single strength grapefruit juice from Florida (1979 - 1980)

	Month packed			
	Nov.	Dec.	Jan.	Feb.
Number samples	15	16	16	8
Average tin conc. (ppm)	125	107	67	51
Median tin conc (ppm)	119	94	68	50
Range (ppm)	75-172	46-180	34-135	34-68
Average pH	3.38	3.36	3.39	3.41
Average age (month)	6.9	5.0	2.5	1.3

A wide range of tin values was observed (34 - 180 ppm) and even after more than 6 months storage under commercial conditions the average tin concentration was 125 ppm. None of the commercial samples exceeded the Codex Alimentarius Commissions (1978) standard of 250 ppm tin.

REFERENCES

Bakal, A. and Mannheim, H.C. 1966. The influence of processing variants of grapefruit juice on the rate of can corrosion and product quality. Israel J. Technol. 4(4): 262.

Benoy, C.J., Hooper, P.A., and Schneider, R. 1971. Toxicity of tin in canned fruit juices and solid foods. Food Cosmet. Toxicol. 9(5): 645.

Bird, D.E. 1980. Estimation of shelf life from tinplate properties. I.T.R.I. Publ. (2nd Int. Tinplate Conf.) 600: 369.

Calloway, D.H. and McMullen, J.J. 1966. Fecal excretion of iron and tin by men fed stored canned food. Am. J. Clin. Nutr. 18: 1.

Capacho-Delgado, L. and Manning, D.C. 1966. Determination of tin by atomic absorption spectroscopy. Spectrochim. Acta. 22(8): 1505.

Codex Alimentarius Commission. 1978. "Report of the Twelfth Session." FAO/WHO, Rome, p. 45.

Debost, M. and Cheftel, J.C. 1979. Tin binding in canned green beans. J. Agric. Food Chem. 27(6): 1311.

Dickinson, D. 1961. The mechanism of corrosion of tinplate cans by fruit. Corrosion Technol. 8: 201.

Fritsch, P., De Saint Blanquat, G., and Derade, R. 1977. Effects of various dietary components on absorption and tissue distribution of orally administrated inorganic tin in rats. Food Cosmet. Toxicol. 15(2): 147.

Hiles R.A. 1974. Absorption, distribution and excretion of inorganic tin in rats. Toxicol. Appl. Pharmacol. 27: 366.

Juliano, P.O. and Harrison, W.W. 1970. Atomic absorption interference of tin. Anal. Chem. 42(1): 84.

Kefford, J.F., McKenzie, H.A., and Thompson, P.C.O. 1959. Effects of oxygen on quality and ascorbic acid retention in canned and frozen orange juices. J. Sci. Food Agric. 10: 51.

Kutzner, J. and Brod, K.H. 1971. Resorption and excretion of tin after oral administration of tin-133. Nuclear Med. 10: 286.

Mannheim, C.H. and Hoenig, R. 1979. Storage changes in canned comminuted orange and lemon as affected by type of can. Confructa. 16: 165.

Nagy, S., Rouseff, R., and Ting, S. 1980. Effects of temperature and storage on the iron and tin content of commercially canned single strength orange juice. J. Agric. Food Chem. 28: 1166.

Nishijuma, M., Ueda, K., Maki, T., Wada, M., Takahashi, S., Kimura, Y., Yamada, T., Nakajima, K., and Tukuda, T. 1971. Heavy metals dissolved in canned foods. 2. Detection of tin and lead from various canned fruits after opening. Tokyo Toritsu Eisei Kenkyusho Kenkyu Nempo. 23: 189.

Price, W.J. and Ross, J.T. 1969. Analysis of fruit juice by atomic absorption spectrophotometry. 1. The determination of iron and tin in canned juice. J. Sci. Food Agric. 20: 437.

Royo-Iranzo, J., Catala, R., and Primo, E. 1970. Tin content in canned orange juice. Int. Fruchtsaftunion, Wiss. - Tech. Komm. 10: 77.

Saguy, I., Mannheim, C.H., and Passy, H. 1973. Role of sulfur dioxide and nitrate on detinning of canned grapefruit juice. J. Food Technol. 8(2): 147.

Effects of Toasting on the Quality of Canned Rice

R. N. SHARP, M. W. KATTAN, and C. Q. SHARP

————————————ABSTRACT————————————

Parboiled long grain milled rice was toasted to assess the effects of
dry heat on the canning quality. Toasting was accomplished by ex-
posing the rice to 170°C for 0, 15, 30, 45 or 60 min. Increased
toasting time resulted in decreased whiteness and yellowness and in-
creased redness, but the intensity of the color change was dimin-
ished by hydration and canning. Toasting time increased the force
required to shear the canned product; however, the rate of retro-
gradation, as measured by amylographic viscosity, seemingly was
unaffected by the toasting treatments. Sensory evaluations indicated
that preferences for flavor and general appearance of the canned
toasted rice were very individualistic.

INTRODUCTION

WAYS TO IMPROVE the utilization of rice are constantly
being explored. In recent years, a method for canning rice
that eliminated the requirement for pre-soaking and pre-
cooking was reported (Sharp et al., 1981). This procedure
was further refined to provide a variety of canned rice
products (Sharp et al., 1982). Rice, as some recipes suggest,
may be toasted to provide color and distinctive taste to
finished food. Baked breads are sometimes toasted also
for the purpose of flavor change and darker color devel-
opment (Knight et al., 1982). Fellers et al. (1983) reported
that toasting by a blast of hot air reduced the stickiness of
medium grain rice. The toasting of rice has also been re-
ported to influence the cooking quality (Sharp et al., 1984).
This study was undertaken to determine the effects of dry
heat on the color, water uptake, texture and sensory panel
acceptability of canned parboiled milled rice.

MATERIALS & METHODS

Product preparation

Parboiled long grain rice of the Lebonnet variety (Riceland
Foods, Inc., Stuttgart, AR) was used throughout this study. Two
hundred grams of rice were evenly distributed onto a 32 × 41
cm preheated tray and placed in a mechanical convection oven at
170°C for 0, 15, 30, 45 or 60 min. After toasting, the rice was
allowed to air cool to room temperature. Rice was processed in 211
× 400 R (unpigmented lacquer used to increase resistance to corro-
sion or decrease bleaching effect of tin plate) enamel metal cans
according to the procedure of Sharp et al. (1982). Eight cans were
processed from each toasting time.

Quality evaluations

A Gardner Color Difference Meter (CDM), calibrated with a
white standard plaque (L = 92.4; a = −1.0 and b = 1.0) was used
to measure the color of the rice both before and after canning.
The sample cup was filled to 1 cm from the top. Color values
recorded before canning were obtained from the dry (non-hydrated)
rice, while the rice after canning was moist. Fifty grams of canned
product and 50 mL deionized water were blended for 90 sec using
an Osterizer blender to give improved precision of color evaluation.
pH readings of the blended samples were recorded. Approximately
10g of drained product were dried in a mechanical convection oven
at 68°C for 48 hr for dry matter determination.

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An untrained seven-member panel evaluated each canned rice
product for texture, flavor and general appearance using a 5 point
hedonic scale. Sensory evaluations were conducted in an open area
setting. Objective texture measurements of the rice products were
obtained using an Allo-Kramer recording shearpress with a 13 blade
compression cell to shear 100g of canned product.

Amylograph and water uptake

Parboiled and toasted parboiled rices were ground to 0.4 mm
using a Cyclone Sample Mill (UD Corp., Boulder, CO). Fifty grams
of the resulting rice flour were suspended in 450 mL of deionized
water. The suspension was heated in a Brabender Visco/amylo/
graph (C.W. Brabender Instruments, Inc., South Hackensack, NJ)
to produce amylographic pasting curves. The temperature was in-
creased at a rate of 1.5°C per min from 25°C to 92.5°C over a per-
iod of 45 min, held at 92.5°C for 15 min, then was returned to
25°C over a 45 min period at a cooling rate of 1.5°C per min. The
following reference viscosities were noted: at 92.5°C, after 15 min
at 92.5°C and upon cooling to 25°C. Pasting temperature (first in-
flection) was observed.

Water uptake ratio as a measure of severity of heat treatment
(water uptake at 60°C divided by water uptake at 96°C) was deter-
mined by the procedure described by Bhattacharya (1979).

Analysis of variance, least significant difference and correlation
coefficients were calculated according to Steel and Torrie (1960).

RESULTS & DISCUSSION

THE COLOR OF RICE has long been claimed to be a
major factor in the acceptability of the finished product.
Tristimulus color values for the rice prior to (nonhydrated)
and after canning are shown in Table 1. The rice became
less white (L value decreased), more red (a value increased)
and less yellow (b value decreased) as the toasting time in-
creased. The canned products were whiter and less red than

Table 1—Tristimulus color values of toasted rice before and after
canning

Toasting time (min)	Tristimulus color values[a]		
	L	a	b
	Before canning		
0	65.7	1.7	23.4
15	57.7	7.7	25.1
30	46.6	12.5	23.0
45	42.0	13.5	20.7
60	40.2	14.1	19.3
LSD @ 5%	0.2	0.3	0.1
	After canning		
0	77.4	−2.9	18.9
15	73.4	−0.5	19.5
30	67.1	2.0	20.7
45	64.1	2.7	20.9
60	60.7	3.6	20.9
LSD @ 5%	1.4	0.3	0.4

[a] Gardner Color Difference Meter value: L — White = 100, black =
0; a — Red if positive, Green if negative; b — Yellow if positive.

were the nonhydrated rices. The degree of yellowness was less for the canned rice than for the nonhydrated except for the 45 and 60 min toasting times. The L and a values for the canned rice may be different from the non-canned rice because of the altered physical state: dry whole kernel before canning, compared to hydrated rice after canning. Regardless of these differences, the greater the degree of toasting, the less white and more red the rice. The b value (yellowness) of the non-hydrated rice decreased as the intensity of the toasting increased, while the b value of the canned rice increased from no toasting to 30 min toasting, but no differences were found between canned rice products toasted for 30, 45 and 60 min.

Table 2 shows the dry matter content (g/100g of rice), shear value and pH of the canned rice product. The dry matter content indicated that the toasted rice imbibed less water than did the nontoasted rice. A toasting time greater than 30 min did not significantly decrease the amount of water imbibed. Although the data are not presented, the dry matter content was indicative of the free liquid present in the can. As a general rule, the lesser the amount of water imbibed by the rice, the greater the force required to shear the rice. Data shown in Table 2 agree with these generalities except the canned product toasted for longer than 30 min continued to increase in force required for shearing although there was no difference in water imbibition.

Statistical differences in the pH of the canned products were not found. The low pH of the finished product was due to pH adjustment and control as specified in the canning method (Sharp et al., 1981). The toasting treatment did not promote kernel changes that significantly affected the final product pH.

The sensory panel did not register preference between products in flavor and general appearance; however, individual panelists showed definite preferences. The lack of agreement among panelists depicted varied individual food choices. Panelists were not asked to discern the differences between the samples, only to give their like or dislike for each sample. Objective texture measurements (shear values) indicated a significant difference between toasting times,

but preference evaluations were so individualistic that differences between samples were overshadowed.

Correlation coefficients were developed between tristimulus color values of the toasted rice (both before and after canning) and selected quality evaluations of canned rice (Table 3). L (whiteness) and a (redness) CDM values were significantly related to shear value, dry matter content and subjective texture values, but were not significantly related to subjective ratings for flavor and general appearance. The redness of the toasted rice was more highly associated with shear value, dry matter content and subjective texture of the canned rice than was the whiteness. The degree of yellowness of the non-hydrated rice was not significantly related to any of the selected quality attributes. All three tristimulus color values of the canned rice were significantly related to shear value and dry matter content. CDM a and b values of the canned products were positively associated with subjective texture. None of the color values was significantly related to subjective flavor and general appearance ratings.

The pitfalls in correlating sensory and objective evaluations (Noble, 1975; Trant et al., 1981) are recognized; therefore, cause and effect associations are not intended in the presentation of these data. The relationships between color development during toasting and factors important to preference warrant reporting these calculations.

Bhattacharya (1979) studied relationships between water uptake and severity of heat treatment during parboiling. Raw rice had a water uptake ratio of 0.04 – 0.05 while the water uptake ratio of severely parboiled rice was as high as 0.50. Water uptake indicated that the nontoasted rice was well parboiled and showed an increase in water uptake ratio with increased toasting times (Table 4). Raw data, from which the ratio was calculated, showed that water uptake at $60°C$ increased and at $96°C$ decreased as rice received more severe heat treatment. These findings are in agreement with those reported by Bhattacharya (1979).

Table 3—Correlation coefficients between tristimulus color values of toasted rice and selected quality evaluations of canned rice

Color value[a]		Dry matter	Texture	Flavor	General appearance
		Before canning			
L	−0.97**	−0.98**	−0.93*	0.05	−0.85
a	0.99**	0.99**	0.95**	−0.03	0.84
b	−0.68	0.69	−0.58	0.20	−0.67
		After canning			
L	−0.98**	−0.95**	−0.86	0.17	−0.86
a	0.96**	0.98**	0.92*	−0.11	0.85
b	0.98**	0.96**	0.97**	−0.04	0.78

[a] Gardner Color Difference Meter value: L — white = 100, black = 0; a — redness; b — yellowness.
*,**Significant at P < 0.05 and 0.01, respectively (df = 3).

Table 2—Effect of time of toasting on the percent dry matter, shear force and pH of canned rice

Toasting time (min)	Dry matter (g/100g of rice)	Shear value (lb)	pH
0	21.7	44	4.4
15	22.9	59	4.4
30	23.7	67	4.3
45	24.0	70	4.4
60	24.1	76	4.3
LSD @ 5%	0.5	2	NS

Table 4—Effect of degree of toasting on the water uptake ratio[a] of rice

Toasting time (min)	Water uptake ratio[a]
0	0.409[b]
15	0.456
30	0.474
45	0.490
60	0.509
LSD @ 5%	0.011

[a] Water uptake 60°C/water uptake 96°C.
[b] Average of triplicate values.

Table 5—Effect of degree of toasting on the viscosity of rice flour

Toasting time (min)	Pasting temp (°C)	Amylographic viscosity (B.U.)[a]		
		92.5°C	92.5°C after 15 min	After cooled to 25°C
0	71[b]	520[b]	465	1165
15	78	185	250	570
30	77	155	240	535
45	77	125	215	500
60	78	110	180	450

[a] B.U. = Brabender Units.
[b] Limited sample size allowed only single determinations.

Malting of Hulless Barley Cultivars and Glenlea (*T. aestivum*) Utility Wheat

T. SINGH and F. W. SOSULSKI

——————————— ABSTRACT ———————————

Malting of hulless barley cultivar, Scout, and Utility wheat, Glenlea, was compared with the hulled barley, Harrington. Hulless barley showed faster steeping rate than either Harrington or wheat cultivars. Gibberellic acid, applied by steeping in 1 ppm solution, and germination for 2-8 days significantly increased α-amylase activity of Scout and Glenlea cultivars and their extract yields were 4-6% higher than Harrington malts. The 2-day Scout malts produced highly viscous extracts. Desirable viscosity (5500 cPs) was obtained from 5-day malts.

INTRODUCTION

IN RECENT YEARS, malting of cereals other than hulled barley has attracted attention (Pomeranz et al., 1973, 1975; Sethi and Bains, 1978; Nout and Davies, 1982; Singh et al., 1983; Singh and Bains, 1984). This is because of economic consideration and local availabilities. Malting of hulless barley has been studied to a limited extent (Ballesteros and Piendl, 1975; Rennecke and Sommer, 1979). The hulless barley cv. Scout and the utility wheat cv. Glenlea are relatively low in price and high in protein and starch contents, and are mainly used as feed grains. The purpose of this study was to compare malting of both hulless cultivars with hulled barley, Harrington; and to characterize concentrated extracts for use as food malts.

MATERIALS & METHODS

TWO-ROW BARLEY, *Hordeum vulgare* L. cv. Harrington (hulled), cv. Scout (hulless), and utility wheat (*Triticum aestivum* L.) cv. Glenlea were obtained from the University of Saskatchewan, Canada. The samples represented commercial seed with 95-98% germination.

Germination energy and water sensitivity

Duplicate samples of 100 kernels each were germinated on Whatman No. 1 filter paper, in 9 cm petri dishes containing 4 and 8 mL water (Briggs et al., 1981) at 15°C for 72 hr. The number of kernels which germinated in petri dishes containing 4 mL water was reported as germination energy, and the number of those found resistant to germination in the presence of 8 mL water, was expressed as water sensitivity.

Steeping characteristics

Ten gram samples were steeped in water at 15°C for 72 hr. The weights of the steeped kernels were noted at various intervals and the increase in weight of each sample was recorded. The percentage of water uptake against steeping time was plotted on semi-log paper.

Experimental malting

Weighed lot (120g) of each variety was steeped in water at 15°C to a moisture content of 44%, adopting the steeping times as shown by the linear curves. The steeping water was changed after every 24 hr, followed by an air-rest of 1 hr. Gibberellic acid was applied

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by finally steeping respective lots in 1 ppm solution for 4 hr. To obtain under-, optimum- and over-modified malts, the lots were germinated for 2, 5 and 8 days at 15°C (R.H., 95%) respectively. The green malts were dried in an air oven at 55°C for 20 hr, followed by kilning at 85°C for 4 hr, and cooling to 20°C. The roots were removed by rubbing with hand, and their weights recorded. Malting losses were expressed on basis of dry matter in the grain.

Extract yield and wort quality

The malt samples were mashed according to the AOAC method (1975). Conversion time was recorded by stirring a drop of the mash with iodine solution on a white porcelain plate. Extract yield was determined from the specific gravity (20°C/20°C) equivalent of the filtered wort from the Plato tables of AOAC (1975). The results are expressed as % extract

Color of the worts was determined using a photo colorimeter and results were expressed as absorbance at 430 nm (AOAC, 1975). Total reducing sugars in the worts (% maltose) were determined by using 3,5-dinitrosalicylic acid reagent (Bernfeld, 1955). Titratable acidity (% lactic acid) was estimated by the method of ASBC (1958), and free α-amino nitrogen (mg L^{-1}) using ninhydrin (Lie, 1973). Modification index was calculated by expressing the soluble N in wort as percent of total N in malt.

Malt extract

The worts were concentrated under vacuum at 35°C (Briggs, 1978) and evaluated for color (10% solution), titratable acidity, reducing sugars and protein (% N x 6.25) contents. The viscosity of extracts was determined at 30°C using the Haake rotoviscometer (RV2) and expressed as centipoise (cP).

Moisture, protein, starch and diastatic power as degrees Lintner (°L) were determined by the AOAC procedures (AOAC, 1975); kernel weight by the ASBC method (1958) and the ash content by the AACC method (1969). Alpha-amylase activity in the malts was determined by the ICC colorimetric method (Perten, 1966) using β-limit dextrin substrate. The results are expressed in Sandstedt, Kneen and Blish Units (SKB)/g.

The samples were analysed in triplicate and results examined statistically by the analysis of variance (Steel and Torrie, 1960) with least significant difference (LSD) as the criteria. The relationship between the characteristics of malts, worts and extracts was determined by calculating coefficients of correlation (r) between the variables.

RESULTS & DISCUSSION

KERNEL WEIGHT of Harrington, despite 10.4% husk, was similar to that of Glenlea wheat (Table 1). It was higher than that of Scout, which contained more protein. However, Glenlea had more starch than Harrington and Scout barleys. Harrington barley showed greater water sensitivity than Glenlea and Scout, which were negligibly sensitive.

Steeping characteristics

During the first 9 hr of steeping, the water uptake of the three varieties was rapid, and afterwords, relatively slow (Fig. 1). The hulless barley consistently absorbed more water for similar steeping times. The samples for malting were steeped to 44-45% moisture by adjusting the time.

Malting loss

There were marked differences in the malting losses, which depended more on the duration of germination,

Cultivar	1000-kernel wt. (g)	Protein (Nx6.25) (%)	Starch (%)	Ash (%)	Germination (%)	WS[a] (%)
Barley						
Harrington-hulled	43.4	14.9	60.4	2.1	98	6
Scout-hulless	38.1	17.2	63.0	2.1	98	1
Glenlea-utility wheat	44.1	15.1	68.5	1.9	95	3

[a] WS = Water sensitivity
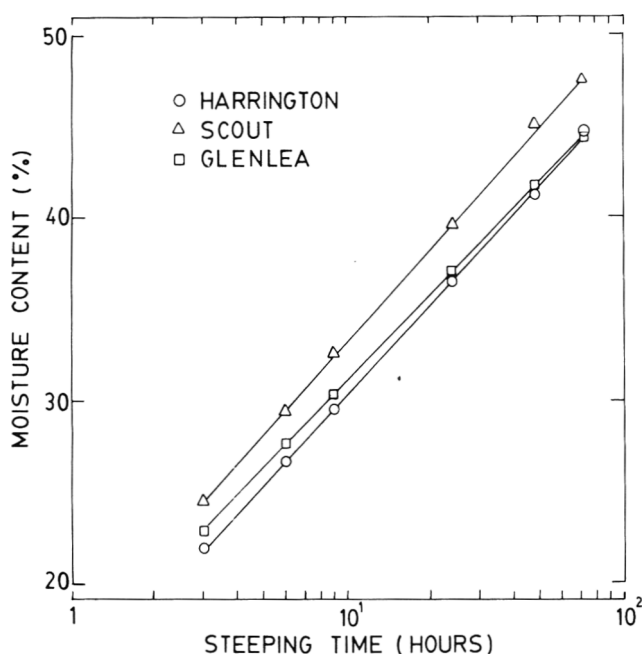
Fig. 1—Water uptake by Harrington and Scout barley and Glenlea wheat kernels during steeping at 15° C.

Fig. 2—Effect of germination and gibberellic acid (GA) on malting loss of Harrington and Scout barley(s) and Glenlea wheat.

being higher in 8-day malts than in 2- and 5-day malts (Fig. 2). Malting loss of gibberellic acid-treated samples was more than that of the control. The hulless barley and utility wheat malting losses were similar to those of Harrington. Malt yields were invariably higher when germination was for 2 days.

Amylolytic activity

The diastatic power of 5-day Harrington malts, with and without gibberellic acid, varied from 93 to 107 degrees Lintner (°L) (Table 2). This was within the range of commercial malts of two-row barley (Briggs et al., 1981). The diastatic power of Scout barley and Glenlea wheat malts was somewhat lower than that of the standard. The diastatic activity of malts was affected more by the duration of germination than by gibberellic acid. The 2-day malts generally showed poor α-amylase activity which increased several fold in 5- and 8-day malts (Table 2). The response of Harrington barley to exogenous gibberellic acid was negligible compared to the increase in the α-amylase activity of Scout and Glenlea malts. Application of gibberellic acid to the 2-day malts significantly increased the α-amylase activity, which was relatively low in case of 5- and 8-day germinated malts.

Extract yield and wort quality

The extract yield of 5-day Harrington malts without gibberellic acid was 80.5% as compared to 83.9% and 82.4%

of Scout and Glenlea malts, respectively (Table 3). The higher extract yield of malts of hulless cultivars than those of hulled barley malts may be attributed to their higher starch contents. Gibberellic acid significantly increased the extract yield of Scout and Glenlea malts accompanied by an increased α-amylase activity.

Conversion time of 2-day malts was longer than the 5- and 8-day malts, possibly due to their lower α-amylase activity (Fig. 3). Glenlea malts took considerably longer time for conversion during mashing than either Harrington or Scout malts. Oloff and Piendl (1978) reported considerably longer conversion times for wheat malts than for barley malts.

The color of Harrington and Scout worts was comparable and lighter, whereas that of Glenlea malts was darker (Fig. 4). The worts of gibberellic acid-treated malts were relatively darker than the controls. The soluble nitrogen in the 2-day malt worts varied from 0.52-0.55% (Table 4), which increased further as germination was extended. The gibberellic acid malt worts contained more soluble nitrogen

Table 2—Effect of germination and gibberellic acid (GA) on diastatic power (DP) and α-amylase (AA) activity of malts, dry bases

Germination (days)	DP (°L)[a]			AA (SKB/g)[b]		
	Harrington	Scout	Glenlea	Harrington	Scout	Glenlea
Control						
2	63	41	52	3.9	2.7	2.3
5	93	79	86	26.9	18.8	19.9
8	103	91	86	39.1	29.2	27.1
GA						
2	84	70	71	7.5	10.0	6.2
5	107	80	92	30.1	28.1	30.4
8	105	92	95	40.0	34.1	39.0
LSD (0.05)	12	12	18	2.4	2.4	3.4

[a] °L = Degree Lintner
[b] SKB = Sandstedt, Kneen, and Blish units of α-amylase

Table 3—Effect of germination and gibberellic acid (GA) on extract yield of malts, dry basis

Germination (days)	Extract yield (%)		
	Harrington	Scout	Glenlea
Control			
2	76.5	80.1	79.6
5	80.5	83.9	82.4
8	80.0	84.2	82.9
GA			
2	78.0	83.7	84.2
5	82.3	85.8	87.6
8	81.2	86.9	86.0
LSD (0.05)	1.7	1.7	2.5

Fig. 3—Effect of germination and gibberellic acid (GA) treatment on conversion time (min) of malt prepared from Harrington and Scout barley(s) and Glenlea wheat.

than controls and two-row barley commercial malts (Briggs et al., 1981). The worts of Harrington, Scout and Glenlea cultivars had about the same amounts of soluble nitrogen, despite differences in their protein contents. It is likely that either lower proteolytic activity/or resistance of Scout protein to proteolysis were responsible for this situation. Pomeranz et al. (1975) reported that with an increase in total protein in barley, the increase in soluble protein was less than the increase in storage protein. A higher proportion of storage protein in Scout barley evidently resulted in lower soluble nitrogen levels in the wort. The reducing sugars in the worts showed negligible differences (Table 4). Titratable acidity values of the worts of gibberellic acid-treated malts were about the same but higher than those of control worts. A statistically significant correlation between titratable acidity and reducing sugar values of the worts was obtained (r = +0.75, p < 0.01). According to Briggs et al. (1981) organic acid are produced during the respiration process from reducing sugars. These acids ultimately find their way in the wort. The amount of free α-amino nitrogen was significantly lower in the worts of Glenlea malts than in the worts of Harrington malts. Worts of gibberellic acid-treated malts had about the same free α-amino nitrogen contents. The relationship between malt protein vs wort α-amino nitrogen was nonsignificant.

The correlation between the values for modification index of worts vs soluble nitrogen (r = +0.64, p < 0.01) and free α-amino nitrogen (r = +0.90, p < 0.01) was statistically highly significant. Modification the malts depended on germination and the application of gibberellic acid (Table 5). The 5- and 8-day Harrington malts, without gibberellic acid, were adequately modified, whereas Scout and Glenlea malts were under- and over-modified, respectively. The
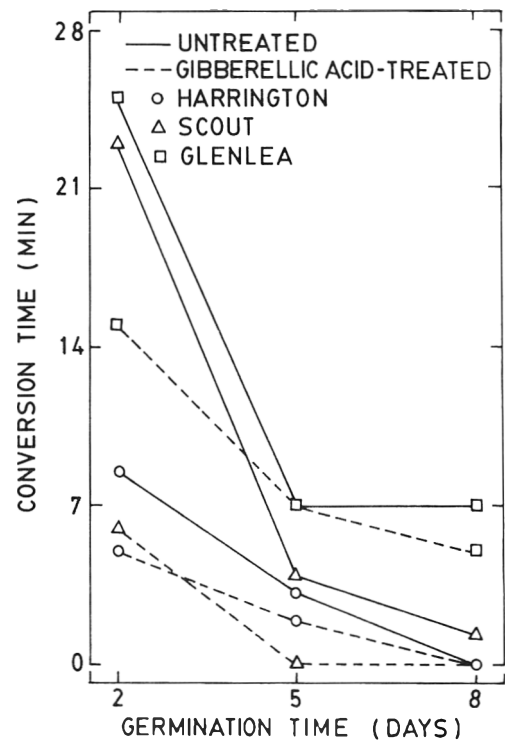
5- and 8-day malts of Harrington and Glenlea wheat were significantly over-modified by gibberellic acid as compared to Scout malts.

Malt extract

The composition of concentrated extract of various malts is shown in Table 6. Increased protein contents, reducing sugars and titratable acidity were the artifacts of concentration of the malt worts. The color of the Glenlea malt extracts was darker than that of Harrington or Scout barley malts (Fig. 5). Wort color values were highly correlated with the color of the extracts (r = +0.96, p < 0.01). The differences in protein contents of the extracts were the results of the degree of modification of malts and free α-amino nitrogen of worts. Highly significant correlations were obtained between protein content of the concentrates and the values for the modification index of malts (r = + 0.97, p < 0.01), and the free α-amino nitrogen of worts (r = + 0.93, p < 0.01).

Table 4—Effect of germination and gibberellic acid (GA) on quality of worts

Germination (days)	Soluble N (%)			TRS[a] as maltose (%)			TA[b] as lactic acid (%)			FAN[c]/L^{-1} (mg)		
	Harrington	Scout	Glenlea	Harrington	Scout	Glenlea	Harrington	Scout	Glenlea	Harrington	Scout	Glenlea
Control												
2	0.55	0.52	0.53	6.3	6.4	6.1	0.08	0.12	0.09	121	114	88
5	0.86	0.84	1.18	7.1	7.2	6.9	0.11	0.14	0.12	183	182	154
8	1.10	1.02	1.13	7.4	7.3	7.0	0.15	0.16	0.13	237	192	157
GA												
2	0.77	0.83	0.99	6.9	7.0	6.7	0.10	0.14	0.11	164	155	144
5	1.29	1.17	1.43	7.3	7.4	7.4	0.18	0.20	0.18	270	216	259
8	1.43	1.50	1.53	7.4	7.6	7.9	0.22	0.25	0.21	320	288	303
LSD (0.05)	0.17	0.17	0.25	0.3	0.3	0.4	0.04	0.04	0.05	24	24	34

[a] TRS = Total reducing sugars.
[b] TA = Titratable acidity.
[c] FAN = Free α-amino nitrogen.

Fig. 4—Effect of germination and gibberellic acid (GA) treatment on color intensities of worts prepared from malts of Harrington and Scout barley(s) and Glenlea wheat.

The extracts of 2-day Glenlea malts without gibberellic acid had lower viscosities than those of similar malts from Harrington or Scout barley (Fig. 6). The varietal differences in the viscosities of the extracts diminished as germination was extended to 5 and 8 days. The desirable viscosity (5500 cPs) for commercial purposes (Anon., 1983) could be obtained from 5-day malt extracts. Exceptionally high viscosities of 2-day barley malt extracts were attributed to undegraded β-glucans released from the endosperm walls by the proteolytic anzymes (Bamforth et al., 1979). Aastrup (1979) reported that β-glucans released during the initial state of malting accounted for the high viscosities of the extracts. Degradation of β-glucans by the enzyme β-glucanases during prolonged germination and during mash-

Table 5—Effect of germination and gibberellic acid (GA) on modification index (MI) of malts

Germination (days)	MI[a]		
	Harrington	Scout	Glenlea
Control			
2	24.1	20.1	21.9
5	38.1	32.2	44.5
8	48.4	38.1	46.4
GA			
2	33.6	31.8	41.2
5	57.3	44.2	58.8
8	61.6	55.6	61.9
LSD (0.05)	7.2	7.2	10.1

[a] $MI = \dfrac{\text{Soluble wort N}}{\text{Total malt N}} \times 100$
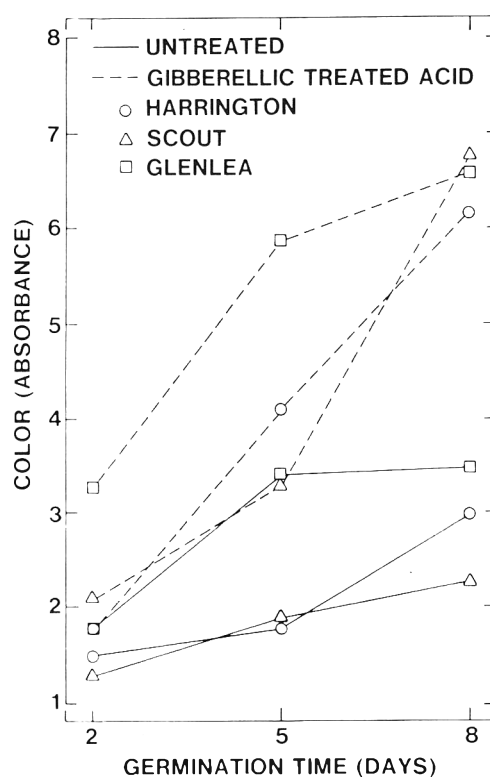
Fig. 5—Effect of germination and gibberellic acid (GA) treatment on color intensities of 10% solution of extracts prepared from malts of Harrington and Scout barley(s) and Glenlea wheat.

Table 6—Effect of germination and gibberellic acid (GA) on quality of concentrated malt extracts

Germination (days)	Protein (Nx6.25) (%)			TRS[a] as maltose (%)			TA[b] as lactic acid (%)		
	Harrington	Scout	Glenlea	Harrington	Scout	Glenlea	Harrington	Scout	Glenlea
Control									
2	3.6	3.4	3.3	58.3	55.3	58.1	0.66	0.99	0.76
5	5.4	5.0	5.8	58.7	59.1	58.2	0.89	1.04	1.05
8	6.9	5.9	6.1	60.2	61.4	57.9	1.22	1.22	1.18
GA									
2	5.0	4.7	5.7	58.5	57.0	59.2	0.87	0.95	1.02
5	7.6	6.7	8.6	58.6	60.1	57.8	1.32	1.36	1.45
8	8.8	8.5	9.3	60.8	62.7	57.0	1.72	1.88	1.60
LSD (0.05)	1.0	1.0	1.4	1.8	1.8	2.5	0.23	0.23	0.32

[a] TRS = Total reducing sugars.
[b] TA = Titratable acidity.
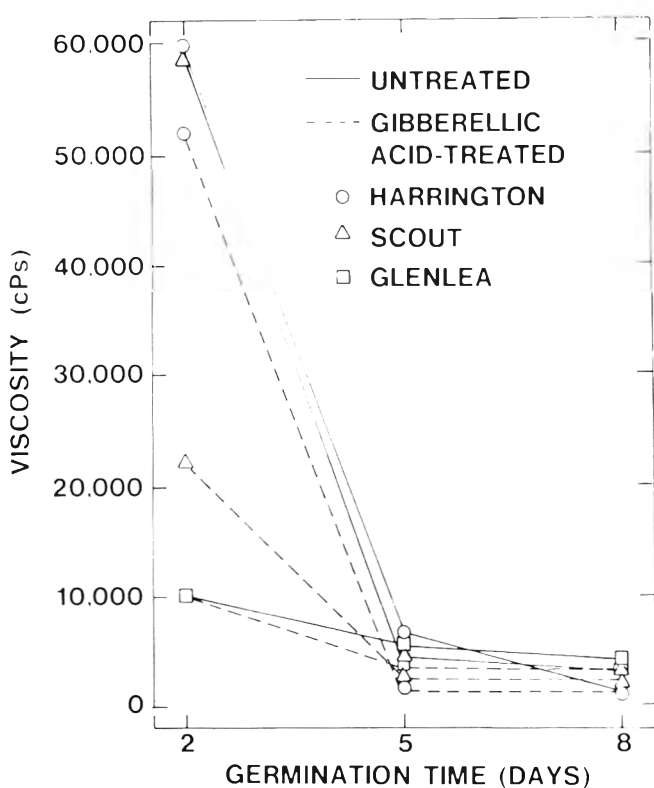
Fig. 6—Effect of germination and gibberellic acid (GA) treatment on viscosity (cPs) of extracts prepared from malts of Harrington and Scout barley(s) and Glenlea wheat.

ing has been stated to reduce the viscosity (Bamforth and Martin, 1983). Concentrates from wheat malts under analogous conditions were not as viscous because of lower β-glucan content in wheat malt than in barley (Prentice et al., 1980). On the other hand, the concentrates of gibberellic acid-treated 2-day Scout malts showed striking reduction in the viscosity. According to Jones (1971), gibberellic acid enhanced the reslease of β-glucanases from the aleurone cells. Possibly, there could be an early release of β-glucanases in the case of Scout barley, producing less viscous concentrates from the 2-day malts.

SUMMARY

MALTS of quality comparable to Harrington (malting barley) can be prepared from hulless cultivars with higher yields. Though the suitability of Scout barley for preparing brewer's malt has to be established, its use for preparing enzymatic, food malts, colored and acid malts should be considered. A concentrated extract for food purposes has practical possibility e.g. the 2-day malts without gibberellic acid but with relatively high viscosity might be advantageous to use in the formulation of gums, candies and other similar products. Glenlea wheat, being higher yielding and lower in price, could be profitably used for malting.

REFERENCES

AACC. 1969, "Cereal Laboratory Methods." Am. Assoc. of Cereal Chem., St. Paul, MN 55121.
Aastrup, S. 1979. The relationship between the viscosity of an acid flour extract of barley and its β-glucan contents. Carlsberg Res. Commun. 44: 289.
Anon. 1983. A correspondence. 1366 Rock Court, Mississauga, Ontario.
AOAC. 1975. "Official Methods of Analysis," 12th ed. Association of Official Analytical Chemists, Washington, DC.
ASBC. 1958. "Methods of Analysis." American Society of Brewing Chemists, Madison, WI.
Ballesteros, G. and Piendl, A. 1977. Effect of malting on proteolytic, amylolytic and phenolic properties of a malting, diastase-rich, feed and naked barley. Brewers Digest Oct.: 36.
Bamforth, C.W. and Martin, H.L. 1983. The degradation of β-glucan during malting and mashing: The role of β-glucanase. J. Inst. Brew. 89: 303.
Bamforth, C.W., Martin, H.L., and Wainwright, T. 1979. A role for carboxypeptidase in solublization of barley β-glucan. J. Inst. Brew. 85: 334.
Bernfeld, P. 1955. Amylase, α-and β-. Methods Enzymol. 1: 149.
Briggs, D.E. 1978. "Barley." Chapman and Hall, London.
Briggs, D.E., Hough, J.S., Stevens, R., and Young, T.W. 1981. "Malting and Brewing Science," Vol. 1. Chapman and Hall, London.
Jones, R.L. 1971. Gibberellic acid- enhanced release of β-1,3-glucanase from barley aleurone cells. Plant Physiol. 47: 412.
Lie, S. 1973. The EBC-ninhydrin method for determination of free alpha-amino nitrogen. J. Inst. Brew. 79: 37.
Nout, M.J.R. and Davies, B.J. 1982. Malting characteristics of finger millet, sorghum and barley, J. Inst. Brew. 88: 157.
Oloff, K. and Piendl, A. 1978. Conventional properties of several malt types. Brewers Digest March: 39.
Perten, H. 1966. A colorimetric method for the determination of alpha-amylase activity. Cereal Chem. 43: 336.
Pomeranz, Y., Standridge, N.N., Robbins, G.S., and Goplin, E.D. 1975. Malting of new wheat cultivars. Cereal Chem. 52: 485.
Pomeranz, Y., Standridge, N.N., Schreck, J.J., and Goplin, E.D. 1973. Rye in malting and brewing. Crop Science 13: 213.
Prentice N., Babler, S., and Faber, S. 1980. Enzymatic analysis of beta-D-glucans in cereal grains. Cereal Chem. 57: 198.
Rennecke, D. and Sommer, W. 1979. Use of naked barley in malting and brewing. Labensmittel Industrie 26: 66.
Sethi, V.B. and Bains, G.S. 1978. Factors influencing the malting quality of Indian wheats. J. Food Sci. Technol. 15: 62.
Singh, T. and Bains, G.S. 1984. Malting of corn: Effect of variety, germination, gibberellic acid, and alkali pretreatments. J. Agric. Food Chem. 32: 346.
Singh, T., Maninder, K., and Bains, G.S. 1983. Malting of Triticum dicoccum (Khapli) wheat: Response to gibberellic acid and use in baking. J. Food Sci. 48: 1135.
Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedure of Statistics." McGraw-Hill Book Co., Inc., NY pp 481.
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Effects of Processing on Aflatoxin Levels and on Mutagenic Potential of Tortillas made from Naturally Contaminated Corn

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─────── ABSTRACT ───────

Naturally contaminated corn containing 127 µg total aflatoxin per kg was divided into kg samples and treated with Ca(OH)$_2$ for making tortillas. The treatments varied in % Ca(OH)$_2$, boiling time, and holding time. Samples were taken after each processing step. Aflatoxin levels and mutagenic activity of acetone extractions of alkaline and reacidified products were measured. All treatments caused a decrease of aflatoxin (up to 46%); however, acidifying products prior to analysis caused reformation of much of the original aflatoxin. All treatments also effected a decrease in the mutagenic potential of the products, except for that of the acidified tortillas which was higher than that of the control corn. The tortilla manufacturing process may not be as effective in aflatoxin destruction as originally surmised.

INTRODUCTION

ALTHOUGH it has long been assumed that the boiling and soaking of corn in limewater during the initial stages of tortilla manufacture would eliminate or greatly reduce the levels of aflatoxin in the final product (Stoloff, 1979), the report that several lots of tortillas purchased in the Mexico City area contained 50 – 200 µg aflatoxin per kg (Martinez, 1979) raised questions about the efficacy of previous testing procedures. In this laboratory, five commonly used tortilla manufacturing methods caused nearly complete disappearance of aflatoxin B$_1$ which had been applied in ethanol to the surface of corn to give a level of 100 µg AFB$_1$ per kg, (Price and Sanchez, 1979). Ulloa-Sosa and Schroeder (1969) showed an apparent 74% decrease in aflatoxin during tortilla manufacture from white corn which had been sterilized, innoculated with *Aspergillus flavus* and then incubated for 5 days. Naturally contaminated corn was not used, and biological tests were not conducted in either study. When tortillas from commercial sources or the naturally contaminated corn used in their manufacture were fed to ducklings, considerable weight loss and liver damage indicative of aflatoxicosis occurred (Martinez, 1979). No adverse effects were found in the ducklings fed noncontaminated corn or tortillas.

In none of the previous studies was the processed product acidified (as would occur in the human stomach) prior to analysis. Reformation of aflatoxin from base-treated media has been shown to occur upon acidification (Parker and Melnick, 1966). This reformation may account for the greater damage to the ducklings in the study by Martinez (1979) than would have been expected from the apparent amounts of aflatoxin in the tortillas.

The purpose of the present study was to determine the effect of five different processing methods on aflatoxin in tortillas made from naturally contaminated corn, the effects of acidification of the tortillas such as may occur in the monogastric stomach, and the mutagenic activity of the products using the Ames microsomal mammalian mutagen assay.

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MATERIALS & METHODS

Tortilla manufacture

Naturally contaminated yellow corn containing an average of 127 µg aflatoxin/kg was obtained from the Univ. of Arizona Experiment Farm in Marana, AZ. The corn was divided into 1 kg samples and treated according to the following conditions: (1) 1 kg corn, 10g Ca(OH)$_2$ (1% by weight of corn), 3L water, boiled for 20 min and allowed to soak overnight (15 hr) (Bazua et al., 1979). (2) Same as #1 except the corn was allowed to soak for 15 hr and then was boiled for 20 min. (3) Same as #1 except the corn was not boiled but allowed to soak for 15 hr. (4) 1 kg corn, 125g Ca(OH)$_2$ (12.5% w/w), 1.6L water, boiled for 1 hr and allowed to cool for 1 hr (Ulloa-Sosa and Schroeder, 1969). (5) 1 kg corn, 7.5g Ca(OH)$_2$ (0.75% w/w), 3L water, cooked for 75 min and allowed to soak for 24 hr.

Corn from treatments 1 – 4 was rinsed thoroughly with water prior to grinding. Corn from treatment #5 was not rinsed. All alkali-treated corn (nixtamal) was ground using an hand-operated Aztec molino Grinder. The dough (masa) (50g) was made into tortillas and cooked on a flat plate for approximately 1 min on each side. Samples of corn (200g each) taken after cooking and soaking were ground and divided into subsamples for subsequent analyses. Samples of masa and tortilla (200g each) were divided into subsamples and analyzed directly. A 50 mL water sample was taken after each processing step.

Chemical analysis

Prior to analysis all samples were randomly coded. Analysis of corn samples was according to Thean et al. (1980) and analysis of water was according to AOAC Official Method 26.A10-26.14(a). All samples were analyzed prior to and after treatment with 0.1N HCl. Aflatoxin content was determined by TLC visual quantitation. All analyses were run in duplicate.

The pH of a 5-g sample homogenized in 50 mL distilled water was monitored after each stage of processing and after each phase of the extraction. The moisture of samples was determined by drying them in a vacuum oven at 60°C until weights were constant. Aflatoxin levels were calculated on a dry weight basis.

Ames test procedure

Prior to testing all samples were randomly coded. Five gram corn samples were twice extracted with acetone, the solvent of choice in AOAC Official Method 26.AO3 for aflatoxin extraction, in a ratio of 1:5 (w/v) according to Felton et al. (1981). The extract was placed in the freezer (−4°C) for 18 hr to allow proteins to precipitate. The extract was filtered, and the acetone was removed using a rotary evaporator. The residue was transferred with a minimum amount of acetone to a 2 dram vial, and the acetone was evaporated using a stream of nitrogen. The final residue was dissolved in 500 µL DMSO for assays.

Salmonella typhimurium strains TA 98 and TA 100 were isolated from frozen cultures to low spontaneous reversion rate colonies and stored according to the procedure of Ames et al. (1975).

Each sample was tested in duplicate for mutagenicity with each strain according to the procedure of Ames et al. (1975). Fifty µL of each extract were applied in DMSO to each culture plate. AFB$_1$ in DMSO in amounts from 10 – 500 µg was used for the standard control plates. Control plates were run with each set of samples. The number of spontaneous revertants on the control plates was subtracted from the number of revertants on each sample plate.

A 3-way statistical analysis of variance was computed; differences between means were tested for significiance using least significant differences at $p < 0.05\%$ (Steel and Torrie, 1960).

RESULTS & DISCUSSION

EACH OF FIVE different methods for tortilla manufacture was effective in reducing aflatoxin levels in naturally contaminated corn. Acidification of samples prior to analysis caused reformation of a major part of the aflatoxin. Mutagenicity of acetone extracts was also decreased by the treatment of the corn with alkali and increased by acidification of the products prior to extraction.

Chemical analysis

Moisture levels of the products of the different stages of the process were corn, 12%, nixtamal, 60%, masa, 60%, and tortillas, 51%.

Levels of aflatoxin found at various stages during manufacture of tortillas from naturally contaminated corn by five different methods are shown in Table 1. Results indicate that there was a significant reduction of aflatoxin by all treatments at most stages of manufacture. The most effective method of treatment was treatment 5, a commercial process used locally. The 20 – 46% decreases in this study are not as great as those reported in previous studies (Price and Sanchez, 1979; Ulloa-Sosa and Schroder, 1969; Martinez, 1979); these differences may be a result of our using naturally contaminated corn in which the aflatoxin was distributed throughout the kernel (Shotwell et al., 1974) where it is somewhat protected from the action of alkali and heat or from oxidation of the open ring form. Destruction of aflatoxin by limewater has been shown to be more effective if the aflatoxin is on or near the surface of the kernel (Price and Sanchez, 1979; Ulloa-Sosa and Schroder, 1969).

Acidification of the samples (such as would occur in the human stomach) prior to analysis indicates that much of the aflatoxin reduction was not permanent, and that acid probably caused a reformation of the aflatoxin by closing the open lactone ring. In every case, the acidified samples had higher levels of aflatoxin, and the differences between the acidified and nonacidified samples were highly significant. Aflatoxin degradation between 23% (method 5) and 46% (method 2) occurred in the final products. Although Parker and Melnick (1966) found nearly 100% reconversion by acidifying an alkali-treated aquous methanol solution of AFB_1, in this experiment, reconversion was less than 100%. This was probably a result of further degradation of the calcium salt of the aflatoxin similar to that seen by Coomes et al. (1966), in which disappearance of afla-

toxin paralleled a decrease in toxicity of autoclaved, contaminated groundnut meal to ducklings. Since levels of aflatoxin in the nixtamal and the masa in all methods were nearly as high as those in the original corn, the cooking of the masa to form tortillas contributed to the greater part of the degradation. Aflatoxin destruction during all stages of these processing method was much less than expected.

Th pH of the limewater in which the corn was soaked and boiled was approximately 13; the pH of the slurries of the nixtamal, masa, and tortilla were approximately 11 for all treatments. This indicates that enough limewater was imbibed by each kernel to raise the pH from neutral to alkaline. Under these conditions, most of the aflatoxin would be expected to be found as the calcium salt and would not be extracted during analysis nor detected by common methods. The pH of the slurried samples to which acid had been added was approximately 6; during analysis the pH dropped to 5 and remained constant thereafter.

Both acidified and alkaline water samples showed less than 5 ppb total aflatoxin. Even though the opening of the lactone ring would increase the solubility of the aflatoxin, Beckwith et al. (1975) found that AFB_1 treated with weak bases bound to corn constituents by electrostatic and/or hydrogen binding interactions. Therefore, it would not be extracted to any extent into the limewater washes and would remain with the corn. Subsequent chemical manipulations during analysis would be sufficient to separate the aflatoxion from the corn constituents.

Mutagenicity

Although the bacterial tester strain, TA 98, is normally used to test frameshift mutagens such as aflatoxin and its metabolic derivatives (Wong and Hsieh, 1976), in this study TA 100, a base pair substitution bacterial tester strain, was equally effective in testing mutagenicity of tortilla acetone extracts.

Acetone extracts taken from the corn and from the alkaline and acidified products showed mutagenic activity using the Ames test with both strains TA 98 and TA 100 (Table 2). These results show that the tortilla-making process was effective in decreasing the mutagenic activity; acidification of the alkali-treated samples caused an increase in mutagenic activity.

Although the mutagenic activity of the acetone extracts of both the masa and tortillas produced by treatment 3

Table 1—Effect of tortilla manufacturing process on aflatoxin levels (μg/kg[a]) in naturally contaminated corn[b]

Treatment	Raw Corn	Cooked		Nixtamal		Masa		Tortilla	
		Alkaline	Acidic	Alkaline	acidic	Alkaline	Acidic	Alkaline	Acidic
(1) Cook 20 min in 0.33% limewater, 15 hr soak, rinse	135a	74c	98c	89b	108b	60s	96c	62bc	93b
(2) Soak 15 hr in 0.33% limewater, 20 min cook, rinse	142a	93b	140a	120a	132a	72cd	117b	58c	77c
(3) Soak 15 hr in 0.33% limewater, no cooking, rinse	145a	uncooked		120a	137a	125a	139a	67ab	84bc
(4) Cook 1 hr in 7.8% limewater, cook 1 hr, rinse	142a	105a	122b	120a	137a	115b	137a	74a	113a
(5) Cook 1 hr, in 0.25% limewater, soak 24 hr, no rinse	142a	89b	120b	91b	132a	77a	137a	38d	110a

[a] Calculations based on dry weight of corn or corn product.
[b] Means within columns sharing the same letter are not significantly different (P < 0.05).

was significantly higher than that of other treatments (likely due to the higher aflatoxin levels of these samples), the mutagenicity of samples from treatment 3 was increased only slightly by acidification. Mutagenicity of the extracts from the other treatments increased substantially and in the case of the tortillas, was significantly greater than extracts from the unprocessed control corn. This could be due to the cooking process and the changes in the corn as a result of the process of making tortillas. Bresanni and Scrimshaw (1958) and Bresanni et al. (1958) found that significant changes in the carbohydrates, vitamins, proteins and specific amino acids (such as serine, leucine, cystine, arginine and glutamic acid) occur in corn during tortilla manufacture. Sugimura and Nagao (1979) found that mutagenic compounds were produced during the cooking of foods. They also showed that pyrolysis of certain amino acids (serine, glutamic acid and tryptophan) yielded compounds which were highly mutagenic to strains TA 98 and TA 100. Spingarn et al. (1983) found that mutagens were formed during the heating of amino acids and sugars under basic conditions comparable to those of tortilla manufacture.

In comparing the number of revertant colonies of the acidified and the nonacidified samples, not only should the increased aflatoxin levels be considered but also the presence of phenolic acids and other compounds (i.e., phytic acid) should be considered. Sosulski et al. (1982) showed that corn contains over 300 ppm phenolic acids which were extracted under acidic conditions. Therefore, the importance of the mutagenic tests on the acidified samples cannot be determined since even more mutagenic substances were extracted from the final products of tortilla manufacture than from the unprocessed original corn.

The number of reversions produced by all of the water samples were not significantly greater than the spontaneous reversions with the Ames Test using both strains TA 98 and TA 100. This indicates that most mutagneic compounds remained in the corn and were not extracted into the water during cooking.

Our results suggest that increasing the amount of $Ca(OH)_2$ during cooking and soaking did not cause a greater reduction of aflatoxin in tortillas. Martinez (1979) found that there was no significant difference in the aflatoxin levels betweenn those tortillas which were processed with 2% $Ca(OH)_2$ and those processed with 10% $Ca(OH)_2$. This effect could be in part due to the solubility of $Ca(OH)_2$

in water (1.85g/L) which decreases when the water is boiled (0.77 g/L). Adding more than these amounts of $Ca(OH)_2$ did not increase the effectiveness of the process and appears to be unnecessary.

The results of this and previous experiments would tend to support the following sequence of events during tortilla processing: As the corn is soaked in limewater and then cooked, aflatoxin is converted to the open-ring form, analysis shows less aflatoxin and the mutagenicity of acetone extracts decreases. During the boiling of the corn in basic solution or subsequent cooking of the tortilla, some mutagenic substances are formed which may be extracted with acetone from acidic solution, and some of the open-ring form of the aflatoxin suffers further degradation, possibly decarboxylation (Coomes et al., 1966). If the corn is not boiled in basic solution, as in Treatment 3, the aflatoxin levels are not reduced to any extent, and the mutagenic activity remains relatively high. Acidification of the final product causes a reformation of much of the non-degraded aflatoxin and an in crease in the quantity of mutagenic substances, including aflatoxin, which are extracted from the products by acetone.

In conclusion, these results show that the processes used in tortilla manufacture reduce levels of aflatoxin in tortillas to some degree. However, because reformation of much of the aflatoxin may occur in the acid, monogastric stomach, the process may not necessarily yield as safe a product to humans as originally presumed (Stoloff, 1979). In the manufacture of tortillas for human consumption, it is more important to choose high quality corn with minimal aflatoxin contamination than to rely upon the process to degrade aflatoxin in contaminated raw material.

REFERENCES

Ames, B.N., McCann, J.R., and Yamaski, E. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mut. Res. 31: 347.
AOAC. 1980. "Official Methods of Analysis," 13th ed, Ch. 26. Association of Official Analytical Chemists, Washington, DC.
Bazua, C.P., Guerra, R., and Sterner, H. 1979. Extruded corn flour as an alternative to lime-treated corn flour for tortilla preparation. J. Food Sci. 44: 940.
Beckwith, A.C., Vesonder, R.F., and Cigler, A. 1975. Action of weak bases upon aflatoxin B_1 in contact with macromolecular reactants. J. Agric. Food Chem. 23: 582.
Bressani, R., Paz y Paz, R., and Scrimshaw, N.S. 1958. Chemical changes in corn during preparation of tortillas. J. Agric. Food Chem. 6: 770.
Bressani, R. and Scrimshaw, N.S. 1958. Effect of lime treatment on in vitro availability of essential amino acids on protein fractions in corn. J. Agric. Food Chem. 6: 773.

Table 2—Number of revertants[a] from acetone extracts of acidified and nonacidified alkali treated corn using Salmonella typhimurium TA98 and TA100[b]

Treatment	TA 98					TA 100				
	Raw corn	Masa		Tortilla		Raw corn	Masa		Tortilla	
		Alkaline	Acidic	Alkaline	Acidic		Alkaline	Acidic	Alkaline	Acidic
(1) Cook 20 min in 0.33% limewater, 15 hr soak, rinse	815	738b	750c	408b	1248	839	977ab	1075	560b	1302a
(2) Soak 15 hr in 0.33% limewater, 20 min cook, rinse	880	561c	827b	356b	1140	820	647bc	927	480b	1190b
(3) Soak 15 hr in 0.33% limewater, no cooking, rinse	1002	957a	989ab	932a	1101	990	862a	901	790a	977c
(4) Cook 1 hr in 7.8% limewater, cook 1 hr, rinse	911	524c	890b	382b	1408	906	809ab	904	498b	1255a
(5) Cook 1 hr, in 0.25% limewater, soak 24 hr, no rinse	862	513c	1061a	400b	1320	895	540c	1029	420b	1196b

[a] Calculations based on dry weight of corn or corn product.
[b] Means within columns sharing the same letter are not signifciantly different (P < 0.05).

Stability of Glutamic Acid and Monosodium Glutamate Under Model System Conditions: Influence of Physical and Technological Factors

A. GAYTE-SORBIER, Ch. B. AIRAUDO, and P. ARMAND

──────ABSTRACT──────

The stability of glutamic acid and monosodium glutamate as regards pH, temperature and oxygen was studied during storage and two thermal processings, the residual products and those which appeared being determined. The initial molecules only change into pyrrolidonecarboxylic acid, never into glutamine nor γ-aminobutyric acid. Preservation is good in all cases at pH 0 and pH 14, but glutamic acid is converted to pyrrolidonecarboxylic acid at intermediate pH values. The change is favored by pH values ranging from 2 – 3, by temperature increase and by oxygen.

INTRODUCTION

FREE GLUTAMIC ACID in sodium salt form is added as a taste-active ingredient to many industrially prepared food products. It exists naturally, however, in varying concentration in free form, in several foods such as chicken, shellfish, crustacea, different vegetables and fruit. In combined form, glutamic acid is also a native constituent of food proteins. This amino acid on the other hand plays an important role at several levels of nitrogen metabolism.

This double aspect of glutamic acid, sometimes as an additive, sometimes as a normal constituent of foods, and its metabolic importance have been reviewed in a book recently published (Filer et al., 1979). They induced us to study the fate of glutamic acid under different storage conditions and during several technological procedures. Wilson and Cannan (1937) observed that glutamic acid was converted to pyroglutamic acid at some pH values, but their work was almost exclusively theoretical. Later, Olcott (1944) showed that autoclaving a protein hydrolyzate changed glutamic acid almost entirely into pyroglutamic acid under certain conditions. Goodban et al. (1953) established that this derivative was produced from glutamine during beet juice processing. It has long been known that vegetables lose free glutamic acid during cooking, as well as during the first 24 hr of refrigerator storage (Hac et al., 1949). It is now recognized in the food processing industry that thermal processing reduces the taste-active effect of added monosodium glutamate if processing takes place after addition. Rice and Pederson (1954), and El Miladi et al. (1959) stressed the pyroglutamic concentration increase in heated and/or stored tomato juices. The same phenomenon was observed in cherries and in some vegetables which had been heated, then stored for 2 yr (Mahdi et al., 1959), in spinach (Lin et al., 1970, 1971), and in carrots (Bibeau and Clydesdale, 1975) after different treatments. The pyroglutamic acid concentration increase was attributed in several of these cases to glutamine conversion, since glutamine concentration decreased accordingly.

It was assumed that the more marked flavor of freshly gathered vegetables might be due to their higher concentration of glutamic acid (Hac et al., 1949) and that the appearance of off-flavors was probably related to the increase in pyroglutamic acid concentration (Rice and Pederson, 1954). Subsequent investigations (Shallenberger and Moyer, 1958; Shallenberger et al., 1959) corroborated that ammonium pyroglutamate added to beet purees gave them bitter, medicinal, or phenolic flavors, similar to those of heated beets. Mahdi et al. (1961) concluded that pyroglutamic acid changed the flavor of various foods when its concentration reached a given level, which varied with the food in question.

The objective of the present work has been to study the different factors influencing the conversion of glutamic acid during its storage and processing, and to identify the conversion products.

MATERIALS & METHODS

SOLUTIONS at 1 g/L and 5 g/L of L-glutamic acid (Merck, ref. 291) and monosodium L-glutamate (Merck, ref. 6445) were prepared in acid and alkaline aqueous dilutions, obtained from normal solutions of hydrochloric acid and sodium hydroxide so as to cover the whole range of pH values from 0 – 14 steps of 1 pH unit. This range is considerably larger than the normal range of food pH values and allowed us to study the stability of glutamic acid in all possible situations, thus extending the practical range into the realm of theory. The solutions were sterilized by filtration through membrane (Millipore, Millex type, ref. SLKA 02505, 25 mm diameter, 0.45 μm porosity) as soon as they were prepared. The effect of the different storage and processing procedures described below was evaluated on each of the solutions.

Storage and processing conditions

In all storage studies, the solutions were stored for 50 days in the dark in sterilized ground-glass stoppered bottles. In order to study the preservation of glutamic acid and monosodium glutamate protected from the air, the solutions were stored at room temperature (20°C) and in a refrigerator (4°C) in completely filled and stoppered bottles. Each determination of residual concentration after a given time necessitated an individual bottle to avoid the entry of air resulting from sampling. The solutions were therefore not entirely oxygen-free, but the influence of air was limited to that of the air dissolved in them. This storage was termed, for convenience sake, "without atmospheric contact." For the study of preservation at room temperature under nitrogen, the dissolved air was eliminated by bubbling nitrogen through the solutions. A nitrogen atmosphere was maintained on the liquid surface before the bottles were stoppered and reconstituted after each sampling. To study the effect of oxygen, storage was similar to that above, except that nitrogen was replaced by oxygen. Finally, to study the effect of air, the solutions were stored in stoppered (in order to avoid microbial contamination) but half-full bottles and with residual air on the liquid surfaces. In each experiment, the time dependent residual glutamic acid or monosodium glutamate concentrations were determined after 1, 2, 3, 5, 10, 15, 20, 30 and 50 days, as were the conversion product concentrations.

Boiling under reflux was done for 15, 30, 60 and 120 min and residual glutamic acid was determined as soon as the solutions had cooled. The conversion products were determined, however, only after 60 min boiling, based on information from the study on the disappearance of glutamic acid. In the autoclaving processing, the solutions were heated to 135°C under 2.7 kg/cm^2 pressure in screw-top glass bottles with bakelite caps and Teflon® seals ("SVL"®, France). Conversion products were determined only after 30 min processing.

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Analytical methods

Glutamic acid and monosodium glutamate were determined by oxidative deamination with L-glutamate dehydrogenase (Bernt and Bergmeyer, 1974). We have reported a critical study of this method (Armand et al., 1976). γ-Aminobutyric acid was separated by ion exchange liquid chromatography according to the Moore and Stein method (Spackman et al., 1958), using ninhydrin. Glutamine was also determined by the same method. Pyroglutamic acid was determined after reaction with hydroxylamine and iron (III) chloride. These methods have been reported elsewhere (Airaudo et al., 1984).

RESULTS

THE RESULTS are summarized in Tables 1 to 3, in which the value 1.00 is arbitrarily attributed to the initial concentration and the other values quoted are the stoichiometric relations of residual glutamic acid (or monosodium glutamate) and of pyroglutamic acid formed.

Glutamic acid without atmospheric contact did not show any conversion at room temperature for 24 hr, whatever the pH (Table 1). A small change began to occur in acid media after 3 storage days and increased between 3

and 5 days. In alkaline media, the glutamic acid concentration remained unchanged for at least 10 days. The excellent preservation of glutamic acid in very acid (pH 0) and very alkaline (pH 14) media should be particularly emphasized. The smallest residual concentrations of glutamic acid were observed after 50 days at pH close to 3 (Table 2). The glutamic acid which disappeared seemed to be almost completely converted into pyroglutamic acid (Table 2).

When glutamic acid was stored at room temperature under nitrogen, its behavior after 50 days was quite similar to that observed in the previous case (Table 2). The preservation was excellent in very acid and very alkaline media. When degradation was observed, glutamic acid was once again converted to pyroglutamic acid.

On the contrary, the conversion at room temperature under oxygen was larger and faster as early as the first days, except in very acid and very alkaline media (Tables 1 and 2). The greatest conversion was observed between pH 2 and pH 3. Another less marked conversion was also observed between pH 6.5 and pH 8.5. The conversion resulted again in the formation of pyroglutamic acid. Very alkaline and

Table 1—Degradation of glutamic acid (initial concentration 1 g/L represented as 1.00) under different storage conditions at room temperature over 30 days

pH	Residual glutamic acid without atmospheric contact							Residual glutamic acid under oxygen					
	Number of storage days							Number of storage days					
	1	3	5	10	15	20	30	2	5	10	15	20	30
0.0	1.00	1.00	0.99	0.99	0.99	0.99	0.98	0.99	0.99	0.99	0.95	0.90	0.90
1.0	1.00	1.00	0.99	0.99	0.97	0.95	0.92	0.99	0.98	0.96	0.91	0.88	0.85
2.0	1.00	1.00	0.98	0.97	0.96	0.94	0.87	1.00	0.98	0.95	0.84	0.65	0.54
3.0	1.00	1.00	0.98	0.97	0.95	0.92	0.85	1.00	0.98	0.98	0.78	0.62	0.51
4.0	1.00	1.00	0.98	0.97	0.95	0.92	0.86	1.00	0.98	0.97	0.84	0.69	0.62
5.0	1.00	0.99	0.98	0.98	0.95	0.93	0.88	0.98	0.98	0.96	0.88	0.68	0.65
6.0	1.00	0.99	0.98	0.98	0.95	0.94	0.89	0.98	0.98	0.95	0.88	0.66	0.62
7.0	1.00	1.00	0.99	0.99	0.96	0.95	0.90	0.98	0.98	0.95	0.82	0.64	0.59
8.0	1.00	1.00	1.00	0.99	0.97	0.95	0.91	0.98	0.98	0.96	0.76	0.66	0.59
9.0	1.00	1.00	1.00	1.00	0.98	0.96	0.93	0.98	0.98	0.96	0.78	0.73	0.65
10.0	1.00	1.00	1.00	1.00	0.99	0.97	0.94	0.99	0.98	0.96	0.85	0.82	0.77
11.0	1.00	1.00	1.00	1.00	0.99	0.98	0.97	1.00	0.98	0.97	0.92	0.90	0.86
12.0	1.00	1.00	1.00	1.00	0.99	0.99	0.98	1.00	0.98	0.98	0.94	0.92	0.88
13.0	1.00	1.00	1.00	1.00	1.00	0.99	0.98	0.99	1.00	0.98	0.96	0.94	0.92
14.0	1.00	1.00	0.99	1.00	1.00	0.99	0.98	0.99	0.99	0.99	0.98	0.95	0.95

Table 2—Conversion of glutamic acid and monosodium glutamate (initial concentrations 5 g/L, represented as 1.00) under different storage conditions after 50 days[a]

pH	At room temperature										At 4°C			
	Without atmospheric contact				Under nitrogen		Under oxygen		With atmospheric contact		Without atmospheric contact			
	a	a'	b	b'	a	a'	a	a'	a	a'	a	a'	b	b'
0.0	0.99	0.00	1.00	0.00	1.00	0.00	0.95	0.02	0.98	0.00	1.00	0.00	1.00	0.00
1.0	0.93	0.05	0.93	0.07	0.94	0.07	0.80	0.20	0.85	0.15	0.97	0.00	0.98	0.00
2.0	0.88	0.10	0.88	0.11	0.90	0.09	0.47	0.53	0.50	0.47	0.95	0.04	0.95	0.05
3.0	0.86	0.10	0.87	0.10	0.90	0.10	0.47	0.54	0.53	0.47	0.95	0.05	0.95	0.05
4.0	0.87	0.10	0.88	0.11	0.90	0.10	0.56	0.43	0.56	0.45	0.95	0.05	0.95	0.05
5.0	0.88	0.10	0.87	0.10	0.90	0.10	0.60	0.40	0.55	0.45	0.95	0.05	0.95	0.05
6.0	0.88	0.10	0.88	0.10	0.90	0.10	0.57	0.43	0.55	0.45	0.95	0.05	0.95	0.05
7.0	0.88	0.09	0.88	0.10	0.91	0.09	0.54	0.42	0.55	0.45	0.95	0.05	0.95	0.05
8.0	0.91	0.08	0.91	0.08	0.93	0.07	0.54	0.43	0.55	0.45	0.95	0.05	0.95	0.05
9.0	0.93	0.07	0.94	0.06	0.95	0.04	0.60	0.41	0.60	0.41	0.95	0.04	0.95	0.05
10.0	0.97	0.04	0.97	0.05	0.99	0.02	0.78	0.24	0.85	0.13	0.98	0.00	0.98	0.00
11.0	0.98	0.02	0.98	0.00	0.99	0.00	0.86	0.12	0.85	0.13	0.98	0.00	0.98	0.00
12.0	0.99	0.00	0.99	0.00	1.00	0.00	0.89	0.10	0.89	0.10	0.99	0.00	1.00	0.00
13.0	1.00	0.00	1.00	0.00	1.00	0.00	0.91	0.08	0.92	0.08	1.00	0.00	1.00	0.00
14.0	1.00	0.00	0.99	0.00	1.00	0.00	0.95	0.01	0.98	0.01	1.00	0.00	1.00	0.00

[a] a — residual glutamic acid; a' — pyroglutamic acid formed from glutamic acid; b — residual monosodium glutamate; b' — pyroglutamic acid formed from monosodium glutamate.

Table 3—Conversion of glutamic acid and monosodium glutamate (initial concentrations 5 g/L, represented as 1.00) during thermal processings[a]

	Boiling processing							Autoclaving processing at 135°C						
	Number of processing minutes							Number of processing minutes						
pH	15	30	60				120	15	30				60	120
	a	a	a	a'	b	b'	a	a	a	a'	b	b'	a	a
0.0	1.00	1.00	0.99	0.00	1.00	0.00	0.98	0.98	0.96	0.02	0.98	0.02	0.96	0.95
1.0	0.94	0.87	0.88	0.12	0.90	0.10	0.78	0.75	0.69	0.22	0.72	0.24	0.68	0.65
2.0	0.94	0.92	0.83	0.18	0.85	0.15	0.71	0.57	0.39	0.64	0.35	0.70	0.31	0.30
3.0	0.97	0.95	0.87	0.15	0.88	0.13	0.77	0.55	0.35	0.69	0.27	0.75	0.22	0.17
4.0	0.99	0.98	0.94	0.07	0.94	0.06	0.89	0.73	0.51	0.53	0.45	0.55	0.35	0.30
5.0	0.99	0.99	0.99	0.00	1.00	0.00	0.97	0.86	0.74	0.25	0.65	0.28	0.59	0.54
6.0	0.99	0.99	0.99	0.00	1.00	0.00	0.98	0.90	0.84	0.10	0.90	0.10	0.81	0.80
7.0	0.99	0.99	0.99	0.00	1.00	0.00	0.99	0.92	0.88	0.04	0.94	0.06	0.86	0.85
8.0	0.99	0.99	0.98	0.01	1.00	0.00	0.97	0.94	0.90	0.05	0.94	0.07	0.85	0.75
9.0	0.99	0.99	0.98	0.01	1.00	0.00	0.96	0.94	0.88	0.12	0.88	0.11	0.72	0.55
10.0	0.99	0.99	0.96	0.03	0.97	0.02	0.95	0.88	0.80	0.17	0.84	0.15	0.58	0.40
11.0	0.99	0.99	0.96	0.03	0.96	0.05	0.94	0.80	0.75	0.17	0.84	0.15	0.60	0.42
12.0	0.99	0.99	0.96	0.02	0.97	0.03	0.95	0.85	0.80	0.13	0.86	0.12	0.70	0.60
13.0	1.00	1.00	0.97	0.01	0.98	0.02	0.97	0.92	0.87	0.07	0.93	0.05	0.84	0.79
14.0	1.00	1.00	0.99	0.00	1.00	0.00	0.99	0.98	0.99	0.00	1.00	0.00	0.98	0.98

[a] a: residual glutamic acid; a': pyroglutamic acid formed from glutamic acid; b: residual monosodium glutamate; b': pyroglutamic acid formed from monosodium glutamate.

very acid media preserved glutamic acid over the 50 days, but less efficiently than when it was stored without atmospheric contact. The same phenomena occured at room temperature in the presence of air, but were a little less marked than in the presence of oxygen (Table 2).

Glutamic acid stored at 4°C without atmospheric contact was better preserved than at room temperature under the same conditions, or under nitrogen (Table 2). It was completely converted to pyroglutamic acid in this case also, when degradation was observed.

Glutamic acid submitted to boiling under reflux remained stable over 60 min in very acid. in almost neutral and in very alkaline media (Table 3). On the contrary, two lability maxima were observed close to pH 11 and pH 2. The conversion resulted only in pyroglutamic acid.

When glutamic acid was autoclaved, it showed a good degree of stability in very acid and very alkaline media. It was a little more stable in the latter case (Table 3). The greatest lability was observed at pH ranging from 2 to about 3.5. Another, less marked, lability range occurred between pH 8 and pH 13. The conversion was greater and faster than that observed in boiling, and the two pH ranges corresponding to the greatest lability shifted a little towards neutrality. All the glutamic acid degraded was converted to pyroglutamic acid only.

Under all conditions, the results obtained with monosodium glutamate were similar to those obtained with glutamic acid under corresponding conditions (Tables 2 and 3), as would be expected *a priori*.

DISCUSSION

THE STUDY was performed on aqueous solutions to determine the individual effect of each factor influencing the degradation of glutamic acid. Foods were considered to be too complex, because several factors may play a role simultaneously and their influences may be complementary or opposing. It seemed to us that only a study of simple media permitted a generalization; the eventual discordances which might be subsequently observed in foods could be attributed to the interaction of several factors or to the interference of other phenomena.

Whatever the storage conditions, the applied processing and the pH values, glutamic acid and monosodium glutamate were only converted to pyroglutamic acid. Conversion to glutamine or to γ-aminobutyric acid was never observed. We could assume *a priori* that glutamine would

not form because there was no ammonia source in the media studied. It seems more interesting to have shown that glutamic acid does not decarboxylate into γ-aminobutyric acid under any of the conditions tested. The similar behavior of glutamic acid and monosodium glutamate were also expected. In alkaline media, the acid forms the salt (more or less completely in the solutions considered, depending on the alkali concentration), and when monosodium glutamate is in acid media, glutamic acid is displaced from its salt by hydrochloric acid (also according to the concentration), because it is weaker (pK_1 : 2.19, pK_2 : 4.25).

The study has shown that glutamic acid was converted to pyroglutamic acid as soon as it was no longer under extreme pH conditions (pH 0 or pH 14). Given that the majority of food products fall into the pH range 4 – 6, a large part of native glutamic acid or monosodium glutamate added as a taste-active ingredient may be converted into pyroglutamic acid when the products are either stored under normal conditions, or boiled, or canned.

CONCLUSION

THE STUDY we conducted allowed us to underline some factors such as the influence of pH and temperature on the glutamic acid and monosodium glutamate stability, or the identity of the conversion product obtained. It should be borne in mind that the conversion to pyroglutamic acid is important in the slightly acid pH range, which corresponds from a practical point of view to the majority of food products.

REFERENCES

Airaudo, Ch.B., Gayte-Sorbier, A., and Armand, P. 1984. Stability of pyroglutamic acid. Influence of physical and technological factors. J. Food Sci. (submitted for publication).

Armand, P., Abello, G., and Gayte-Sorbier, A. 1976. Méthodes de dosage, du glutamate de sodium. II. Potages en boîte, sauces, plats cuisinés, assaisonnements et arômes divers. Ann. Fals. Exp. Chim. 69: 545.

Bernt, E., and Bergmeyer, H.U. 1974. L-Glutamate. Determination with glutamate deshydrogenase, diaphorase and tetrazolium salts. In "Methods of Enzymatic Analysis," (Ed.) H.U. Bergmeyer. p. 1704. Verlag Chemie, Weinheim, and Academic Press, New York.

Bibeau, T.C. and Clydesdale, F.M. 1975. Organic acid profiles of thermally processed carrot puree. J. Milk Food Technol. 38: 518.

El Miladi, S.S., Gould, W.A., and Clements, R.L. 1959. Heat processing effect on starch, sugars, proteins, amino acids, and organic acids of tomato juice. Food Technol. 23: 93.

Filer, L.J. Jr., Garattini, S., Kare, M.K., Reynolds, W.A., and Wurtman, R.J. 1979. "Glutamic Acid: Advances in Biochemistry and Physiology." Raven Press, New York, NY.

Nucleic Acid, Fiber and Nutrient Composition of Inactive Dried Food Yeast Products

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─────────── ABSTRACT ───────────

Nucleic acid, amino acid, dietary fiber, mineral and vitamin composition of six commercial inactive dried food yeasts was studied. Products A and B (autolyzed extract) were produced from *Saccharomyces cerevisiae* grown on cane- and/or beet molasses. Four products were produced from *Candida utilis* grown on calcium lignosulfate/wood sugars (C), sulfite waste liquor (D) and ethyl alcohol (E and F, autolyzed form). Levels of nucleic acids, protein, most amino acids, folacin and panthothenic acid were higher in *C. utilis* products than in *S. cerevisiae* products. Differences between dietary fiber, most minerals and biotin contents of the two species of yeast were, however, small. Autolysis and/or growth on different substrates affected levels of nucleic acids, dietary fiber and most nutrients.

INTRODUCTION

HYPERURICEMIA and associated clinical conditions such as gout, hyperuricemic nephropathy and renal stones may be due to an imbalance in the endogenous production or excretion of uric acid (Gutman and Yu, 1965; PAG, 1975). Various dietary factors such as high intake of nucleic acids (Waslien et al., 1968; Edozien et al., 1970), protein (Bien et al., 1953; Bowering et al., 1969) and fat (Oeryzlo, 1965) have been reported to elevate blood uric acid levels. Among the dietary factors that influence blood uric acid levels, dietary nucleic acids have the greatest effect (Waslien et al., 1968; PAG, 1975).

It has been suggested that the safe limit of nucleic acids from single cell protein in the diet is 2g per day and that the total nucleic acids from all dietary sources should not exceed 4g per day (PAG, 1975). In recent years increased use of inactive food yeasts for functional and nutritional purposes has been promoted (Protein Update, 1978). Inactive dried food yeasts and their autolysates are used as functional ingredients in a variety of food products such as prepared meats, bakery products, baby foods, simulated meat products, salad dressings, cheese products and spreads, soups, sauces, snacks, and many other food products. Numerous yeast preparations are also offered for sale as food supplements in Health Food Stores. Due to their low cost, protein rich products from yeast and yeast-based coprecipitates have been commercially promoted for various nutritional applications (Protein Update, 1978).

The Health Protection Branch is charged with the responsibility of evaluating acceptability of new (yeast) products as food ingredients in Canada on an on-going basis. Information on composition and nutritional value of the commercially available food yeasts will facilitate the evaluation of new yeast products by the Health Protection Branch. In the present investigation, nucleic acid, fiber and nutrient (protein, amino acids, minerals and vitamins) composition of six commercial inactive dried food yeast products was studied. These yeast products were produced

from two organisms (*Candida utilis; Saccharomyces cerevisiae*) grown on different substrates.

MATERIALS & METHODS

THE SIX YEAST PRODUCTS, which were obtained (during November and December, 1981) from commercial sources, are described in Table 1. Two yeast products (A and B) were produced from *Saccharomyces cerevisiae* which was grown on cane-and/or beet molasses. The remaining four yeast products were produced from *Candida utilis* which was grown on calcium lignosulfate/wood sugars (C), sulfite waste liquor (D) and ethyl alcohol (E and F). Product B was an autolysed extract of *Saccharomyces cerevisiae* while product F was an autolysed product of *Candida utilis* grown on ethyl alchohol. The yeast products were analysed on an "as is" basis in duplicate for moisture, total nitrogen, amino acids; in triplicate for ash, minerals, and in quadruplicate for purines, pyrimidines, dietary fiber and vitamins.

Determination of nucleobases and nucleic acids

The hydrolysis of yeast nucleic acids into bases was carried out with 11.6N perchloric acid ($HClO_4$) for 1 hr at 100°C (Marshak and Vogel, 1951). Yeast samples (100 mg) were placed in glass test tubes (15 x 150 mm) and 3 mL 11.6N perchloric acid were added. The test tubes werer then covered (with Parafilm) and the mixture was agitated and heated at 100°C for 1 hr in a steam bath. After cooling, the mixture was adjusted to pH 4.0 with NH_4OH and made up to 25 mL with distilled water. The samples were then filtered through 0.2 μ millipore filter and analyzed for nucleobases by a high performance liquid chromatography (HPLC) procedure developed in our laboratory. Purines (adenine, guanine, hypoxanthine and xanthine) and pyrimidines (cytosine, 5-methyl cytosine, uracil and thymine) were separated isocratically using a Waters HPLC System equipped with a Perkin Elmer HS 5μ C18 reverse-phase column. The mobile phase consisted of 0.3% acetonitrile in water, and the pH was adjusted to 4.0 with phosphoric acid. The flow rate was 0.8 mL/min and detection was performed at 254 nm.

Total nucleic acids were calculated by multiplying the nucleic acid (purine + pyrimidine) nitrogen with a factor of 9 (PAG, 1975).

Determination of nitrogen and amino acids

Total nitrogen was determined by the macro-kjeldahl method. Amino acids were determined by ion-exchange chromatography on the Beckman 121M amino acid analyzer using three hydrolyses, 6N HCl hydrolysis for all amino acids except sulphur amino acids and tryptophan, performic acid + 6N HCl hydrolysis for sulphur amino acids, and 4.2 N NaOH hydrolysis for tryptophan (Sarwar et al., 1983).

Determination of dietary fiber

Dietary fiber was determined by the method of Englyst et al. (1982) using the AB procedure. The hydrolyzed sugars were analyzed on a gas chromatograph (Hewlett Packard 5880A) equipped with a flame ionization detector. The glass column (2.1 m x 2 mm i.d.) was packed with Supelcoport 100/120 mesh coated with 3% SP 2330. Column temperature was 225°C after 4.5 min at initial temperature (185°C) and a rate of increase of 10°C per min. Injection and detector temperatures were 245 and 250°C, respectively. Data were handled with a GC terminal level IV. Resistant starch represented less than 1% of the sample dry weight. This method does not recover lignin. However, no lignin was found using the methods of Goering and Van Soest (1970) as applied by Mongeau and Brassard 1982). Uronic acid was determined by the method of Scott (1979) using colorimetry.

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Table 1—Origin, substrate and moisture content of inactive dried food yeast products tested[a]

Product	Organism	Substrate	Moisture (%)
A	*Saccharomyces cerevisiae*	Cane- and/or beet molasses	2.9
B (Autolyzed extract)	*Saccharomyces cerevisiae*	Cane-and/or beet molasses	3.4
C	*Candida utilis*	Calcium lignosulfate/wood sugars	5.1
D	*Candida utilis*	Sulfite waste liquor	6.0
E	*Candida utilis*	Ethyl alcohol	7.4
F (Autolysed)	*Candida utilis*	Ethyl alcohol	5.1

[a] Products A and B were purchased from GB Fermentation Industries Inc., Montreal, Quebec, Canada. Product C was purchased from Harrison and Crosfield (Canada) Ltd., Toronto, Ontario, Canada. Product D was kindly provided by the Griffith Laboratories Ltd., Scarborough, Ontario. Products E and F were purchased from UF_ Foods Inc., Rexdale, Ontario. The names of the suppliers mentioned here are only for purposes of identification and do not imply approval or recommendation of the company by the Health Protection Branch to the exclusion of others.

Determination of ash and minerals

The ash content was determined by the AOAC (1980) procedure. For the determination of minerals, 3-5g of each sample were weighed into 50 mL beakers in triplicate, dried overnight in an oven at 105°C and then ashed in a muffle furnace at 450°C using concentrated nitric acid as an oxidizing agent to obtain white ash. The ash was dissolved in 6 mL 25% concentrated HCl and diluted to 40 mL. The solutions were analyzed for iron and zinc by atomic absorption spectrophotometry, using a Perkin Elmer 5000 system, according to the manufacturer's recommendations. Calcium, magnesium, phosphorus, sodium, potassium, copper and manganese were determined by inductively coupled plasma emission spectroscopy using a Perkin Elmer ICP/6000 system according to the manufacturer's recommendations.

Determination of vitamins

Folacin acitivity was assayed microbiologically with *Lactobacillus casei* 7469 according to Herbert and Bertino (1967). Dehydrated "Difco" assay medium (Difco Laboratories, Detroit, Michigan) was used at recommended strength. Incubation was carried out at 37°C for 22 hr and the turbidity was measured at 660 nm. Total panthothenic acid was determined microbiologically with *L. plantarum* ATCC 8014 as described by Zook et al. (1956) using Difco AOAC-USP assay medium. Incubation was carried out at 37°C for 22 hr and the turbidity was measured at 660 nm. Biotin activity was assayed microbiologically with *L. plantarum* ATCC 8014 (Wright and Skeggs, 1944) using Difco-Biotin assay medium. The assay tubes were incubated at 37°C for 22 hr and measured turbidimetrically at 660 nm.

Analysis of data

Arithmetic means were calculated for all the constituents studied in this investigation. The data for purines, pyrimidines, nucleic acid nitrogen, total nucleic acids, and vitamins were subjected to analysis of variance and Duncan's multiple range test (Duncan, 1955).

RESULTS & DISCUSSION

Nucleobases and nucleic acids

Separation of the 8 nucleobases was completed in about 12 min (Fig. 1) The bases were also subjected to hydrolysis with 11.6N perchloric acid for 1 hr at 100°C. The recoveries of cytosine, uracil, 5-methyl cytosine, guanine, thymine and adenine after the hydrolysis were 101, 99, 99, 102, 91 and 99%, respectively. Efficiency of the method of determination of nucleic acid nitrogen was tested with
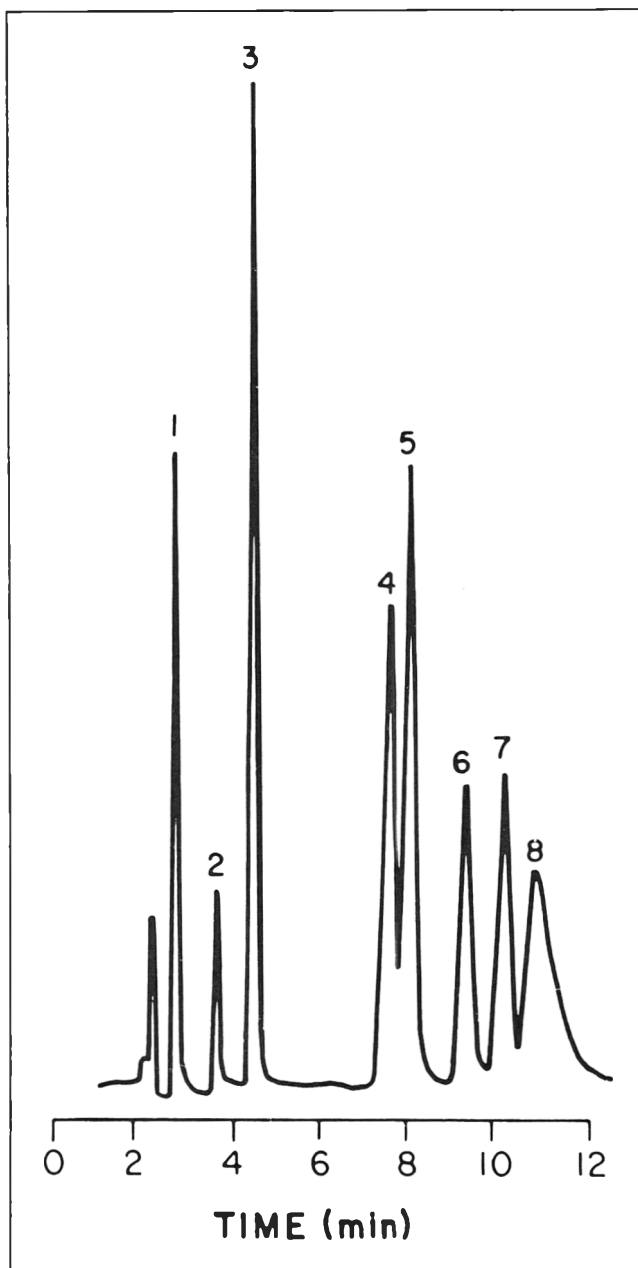
Fig. 1—Chromatogram of standard nucleobases (absorbance at 254 nm, 0-0.05 AUFS). Peaks: 1 = cytosine, 2 = 5-methylcytosine, 3 = uracil, 4 = guanine, 5 = hypoxanthine, 6 - Xanthine, 7 = thymine, 8 = adenine.

yeast RNA and calf thymus DNA (obtained brom Sigma Chemical Co.). The recoveries of nitrogen from yeast RNA and calf thymus DNA were 97 and 98%, respectively.

Clifford and Story (1976) used HPLC with cation exchange columns for the determination of purines in foods, while Hartwick and Brown (1976) introduced the use of (more efficient) reverse-phase HPLC for anlyzing standards of nucleosides and their bases. Tiemeyer et al. (1981) applied the reverse phase HPLC method for the detailed characterization of nucleic acid components in single cell proteins, in which lengthy chemical and enzymatic treatments were used to hydrolyse nucleic acids to nucleotides and free bases. The method reported in this investigation is simple and rapid, and has been successfully applied for the quantitative determination of purines and pyrimidines in meats and some other food products in our laboratory.

Purine and pyrimidine and nucleic acid contents of the yeast products tested in this investigation are shown in

Table 2—Purine, pyrimidine and total nucleic acid content of inactive dried yeast products ("as is" basis)[a]

Product	Adenine mg/100 g	Guanine mg/100 g	Cytosine mg/100 g	Uracil mg/100 g	Total, nucleic acid N NAN, %	Nucleic acids NANX 9, %
A	707[d]	624[f]	344[e]	457[e]	0.900[d]	8.10[d]
B	884[b]	925[b]	436[c]	631[c]	1.209[b]	10.87[b]
C	908[b]	943[g]	524[g]	680[b]	1.276[g]	11.47[g]
D	944[g]	912[c]	499[g]	711[g]	1.279[g]	11.50[g]
E	947[g]	801[d]	469[b]	670[b]	1.207[b]	10.86[b]
F	781[c]	656[e]	399[d]	554[d]	1.000[c]	9.00[c]

[a] Only traces of thymine were detected.
[b-g] Means within the same column bearing different letters are significantly (P < 0.05) different.

Table 2. The six yeast products contained 8.1-11.5% total nucleic acids (Table 2). Product A (S. cerevisiae) had lower levels of individual nucleobases and total nucleic acids than products C, D or E (C. utilis). Product E (C. utilis grown on ethyl alcohol) was lower in total nucleic acids than products D and C (C. utilis grown on sulfite waste liquor and calcium lignosulfate/wood sugars). Autolysis produced different effects on nucleic acid of the two species of yeasts. Product B (autolyzed extract of S. cerevisiae) was higher in individual nucleobases and total nucleic acids than product A but product F (autolyzed form of C. utilis) was lower in individual nucleobases and total nucleic acids than product E. Nucleic acid of single cell proteins has been reported to vary according to the organism and conditions of growth or propagation (Litchfield, 1983). Nucleic acid of S. cerevisiae grown in different media ranged from 4.0-12.9% (Waldron and Lacroute, 1975).

Nitrogen and amino acids

The six yeast products contained 7.44-9.32% total nitrogen (Table 3). Almost all (94-98%) of the nitrogen was recovered as amino acid nitrogen plus nucleic acid nitrogen (Tables 2 and 3). Small amounts of total microbial cell nitrogen have been reported to be present in the form of glucosamine, galactosamine, choline, etc. (Bressani, 1968). Nucleic acid nitrogen constituted 12-16% of the total nitrogen in the yeast products (Table 3). Results of several studies have indicated that up to 20% of the Kjeldahl-reactive nitrogen in the cellular fractions is not protein nitrogen (Bressani, 1968; Kihlberg, 1972).

All the yeast products were high in threonine and lysine but contained low levels of sulphur amino acids and tryptophan (Table 4). Similar observations about the amino acid composition of 8 yeast species were made by Martini et al. (1979). Products A and B (S. cerevisiae) contained lower levels of most amino acids (Table 4) and true protein (Table 3) than products D, C, E and F (C. utilis). True protein (amino acid residue) was calculated as the sum of amino acids minus the elements of water (Tkachuk, 1969). Product D (C. utilis, grown on sulfite waste liquor) contained more protein and (most) amino acids than other forms of C. utilis grown on different substrates such as product E and C (Tables 3 and 4). The lower levels of protein and (most) amino acids in product B compared to A, and in F compared to E suggested that autolysis had decreased the contents of protein and most amino acids in both the species of yeast.

True nitrogen-to-protein conversion factors for the yeast products were calculated to be 5.62-5.81 (with an average of 5.73). As expected, these factors were substantially lower than the commonly used nitrogen-to-protein conversion factor of 6.25.

Dietary fiber

The dietary fiber contents of all yeast products except product B range drom 15.42-17.79% (Table 5). Product B

Table 3—Nitrogen content of inactive dried yeast products ("as is" basis)

Product	Total nitrogen %	Amino acid nitrogen %	Amino acid residue[a] (true protein, %)	N-to-protein conversion factor[b]
A	7.44	6.26	36.36	5.81
B	7.42	5.79	32.57	5.62
C	8.72	7.11	40.63	5.71
D	9.32	7.65	43.63	5.70
E	8.43	7.07	40.55	5.73
F	8.07	6.57	38.08	5.80

[a] Sum of anhydrous amino acids.
[b] Calculated by the method of Tkachuk (1969).

Table 4—Amino acid content of inactive dried yeast products ("as is" basis)

Product	A	B	C	D	E	F
Amino acids, %						
Arginine	2.16	2.07	2.66	2.90	3.13	2.87
Histidine	0.88	0.79	0.99	1.06	0.99	0.94
Isoleucine	2.23	1.65	2.69	2.99	2.31	2.34
Leucine	3.13	2.45	3.93	4.20	3.59	3.36
Lysine	3.34	3.06	3.93	4.16	3.55	3.25
Methionine	0.70	0.46	0.78	0.86	0.61	0.61
Cystine	0.57	0.50	0.51	0.60	0.46	0.36
Phenylalanine	1.78	1.42	2.21	2.36	2.04	1.93
Tyrosine	1.57	1.32	1.92	2.06	1.81	1.73
Threonine	2.14	1.85	2.56	2.61	2.59	2.50
Tryptophan	0.49	0.38	0.56	0.61	0.47	0.51
Valine	2.35	2.25	2.73	2.86	2.65	2.52
Alanine	2.73	2.58	3.03	3.41	2.85	2.69
Aspartic acid	4.44	3.95	4.64	4.89	4.27	4.13
Glutamic acid	7.48	5.77	6.78	7.36	8.71	7.97
Glycine	1.88	1.88	2.23	2.42	2.12	2.01
Proline	1.39	1.45	1.66	1.78	1.50	1.41
Serine	2.09	1.76	2.27	2.43	2.41	2.28
Ammonia	0.86	0.83	0.99	1.06	0.91	0.77

(autolyzed extract of S. cerevisiae) contained only 3.06% dietary fiber (0.07% cellulose and 2.99% noncellulosic polysaccharides). Product F (autolyzed form of C. utilis) contained only about 50% of the cellulose present in E but the differences between the levels of noncellulosic polysaccharides were small. These observations would suggest that the preparation of the autolyzed extract eliminated most of the fiber polysaccharides in the case of S. cerevisiae. Autolysis reduced the level of cellulose in the case of C. utilis but had no effect on the noncellulosic polysaccharides. Noncellulosic polysaccharides constituted the main fiber fraction in all the yeast products (Table 5). Hydrolysis of the fiber polysaccharides of product B yielded

only mannose. In other yeast products, the noncellulosic polysaccharides were made up of mannose, glucose and some arabinose (data not shown). Comparison of products A and B showed that autolysis removed all the glucose and arabinose, and about half of the manoose from the fiber polysaccharides of *S. cerevisiae.* Comparison of product F with E showed that autolysis removed 30% of the glucose and arabinose but none of the mannose from the fiber polysaccharides of *C. utilis.* It seems that the effect of autolysis on the dietary fiber of yeasts is specific to each species or substrate.

The cellulose contents of the nonautolyzed yeast products (Table 5) were in agreement with those reported for similar products by Salo (1977). However, Salo (1977) reported higher hemicellulose than reported in this investigation (Table 5). The difference in hemicellulose may be due to the methods used for fiber analysis.

Ash and mineral nutrients

Ash (Table 6) of product B was very high because of the addition of 37% salt by the manufacturer. The slightly higher values for products E and F were probably due to higher sodium.

From the mineral data, it is evident that the variations in the Mg, P, K, Fe, Zn and Cu (the ratios between the maximum and minimum values being about 20 or less) of the yeast products were relatively less than in Ca and Mn (Table 6). The ratio between the maximum and minimum values for sodium was over 700 but if product B, to which about 150 mg/g sodium was added, is omitted the ratio was less than 5. This indicates that the metabolism of Mg, P, K, Fe, Zn and Na is well regulated and also that there was no contamination of the yeasts with these minerals during growth or subsequent processing. The maximum to minimum ratios for Ca and Mn were about 200 or more, showing wide variations in the levels of the minerals in the yeast products (Table 6). These could have been picked up from the substrates used such as calcium lignosulphate/wood sugar, sulphite waste liquor, and cane and/or beet molasses (which may contain high levels of one or of both minerals).

The differences in Ca and Mn could also be due to the degree of washing before the products were dried.

Products E and F (*C. utilis* grown on alcohol) had similar levels of all nutrients, although the latter was an autolyzed product (Table 6). A comparison of proudcts C and D (*C. utilis,* grown on similar substrates namely calcium ligno-sulphate/wood sugars and sulphite waste liquor) revealed that except for Ca and Mn, all other elements were present at comparable levels (Table 6). The difference between the levels of Ca and Mn could be due to the substrates or due to contamination during processing especially in the case of Mn. When the composition of products A and B (*S. cerevisiae,* grown on cane- and/or beet molasses) was compared, sodium in B was much higher because salt was added to this product by the manufacturer. Whether the lower levels of Mg and Mn in product B were due to autolysis, is not certain because a similar loss was not observed in F, an autolyzed product, in relation to E (a comparable nonautolyzed product).

The proudcts from two organisms used (*S. cerevisiae* and *C. utilis*) did not vary appreciably in the levels of P, K and Zn. The concentrations of the remaining minerals varied widely because of factors such as substrate, intentional additive or processing. If the extreme values were omitted, the levels of Ca, Mg, Na, Fe, Cu and Mn in the products derived from the two organisms were also similar.

Vitamins

The folacin, pantothenic acid and biotin contents of the yeast products are shown in Table 7. Total folacin in the *S. cerevisiae* yeast products (22.4-36.2 μg/g) was lower than in *C. utilis* yeast products (26.5-75.2 μg/g). For each yeast species, the autolyzed products showed significantly lower values (Table 7), indicating that autolysis reduced initial amounts of folacin present in the respective yeasts. Product C (*C. utilis,* grown on calcium ligno-sulfate/wood sugars) had the highest folacin. The range of values for folacin in the yeast products (Table 7) was similar to that reported for Baker's dry, active, and Brewer's debittered yeasts (Perloff and Butrum, 1977).

Similarly, pantothenic acid in the *S. cerevisiae* yeast products (106-127 μg/g) was lower than in *C. utilis* yeast products (126-291 μg/g). When the products from yeasts grown on identical substrates were compared, it was evident that autolysis lowered panthothenic acid level (Table 7). Highest panthothenic acid was found in product E, (*C. utilis,* grown on ethyl alcohol). The panthothenic aicd values for the yeast products (Table 7) were in the range of values published for Baker's dry, active, Brewer's debittered and Torula yeasts (Orr, 1969).

Biotin of the products ranged from 0.84-1.85 μg/g for both species of yeast. There were no consistent effects of types of yeast products and of autolysis on biotin. However, yeasts grown on cane and/or beet molasses or wood sugars, contained more biotin than the yeasts grown on sul-

Table 5—Dietary fiber content (%) of inactive dried yeast products ("as is" basis)

Product	Cellulose	Noncellulosic polysaccharides	Uronic acid	Total fiber
A	4.84	11.45	tr[a]	16.29
B	0.07	2.99	tr	3.06
C	4.82	12.97	tr	17.79
D	4.23	11.19	tr	15.42
E	5.49	12.24	tr	17.73
F	2.47	12.95	0.03	15.45

[a] Traces.

Table 6—Content of mineral nutrients in inactive dried yeast products ("as is" basis)

Product	Ash %	Ca	Mg	P	Na	K	Fe	Zn	Cu	Mn
		mg/g					μg/g			
A	5.8	0.60	1.56	36	0.54	28	70	70	4	4
B	42.8	0.18	0.18	48	170[a]	18	10	80	0.5	0.2
C	5.9	4.64	2.36	58	0.23	22	120	100	4	27
D	5.0	0.89	1.83	55	0.50	19	100	80	12	153
E	7.5	0.05	2.62	68	1.00	33	110	70	1	5
F	7.7	0.09	2.47	69	1.04	33	50	70	1	5

[a] 148 mg/g of sodium added by manufacturer.

Table 7—Folacin, pantothenic acid and biotin in inactive dried yeast products ("as is" basis)

Product	Vitamin content, µg/g of product		
	Total folacin	Pantothenic acid	Biotin
A	36.2[c]	127[cd]	1.85[a]
B	22.4[d]	106[d]	1.34[b]
C	75.2[a]	151[bc]	1.30[b]
D	53.4[b]	126[cd]	0.84[c]
E	52.8[b]	291[a]	0.85[c]
F	26.5[d]	173[b]	1.18[b]

[a-d] Means within the same column bearing different letters are significantly (P < 0.05) different.

fite waste liquor or ethyl alcohol. Highest biotin was found in product A (*S. cerevisiae*, grown on cane and/or beet molasses). The biotin values of the yeast products (Table 7) were lower than the value of 2.0 µg/g published for dried Baker's yeast (McCance and Widdowson, 1978).

REFERENCES

AOAC. 1980. "Official Methods of Analysis," 13th ed, 14.006. Association of Official Analytical Chemists, Washington, DC.
Bien, E.J., Yu, T.F., Benedict, J.D., Gutman, A.B., and Stetten, D.W. 1953. The relationship of dietary nitrogen consumption to the rate of uric acid synthesis in normal and gouty man. J. Clin. Invest. 32: 778.
Bowering, J., Calloway, D.H., Margen, S., and Kaufmann, N.A. 1969. Dietary protein level and uric acid metabolism in normal man. J. Nutr. 100: 249.
Bressani, R. 1968. The use of yeast in human food. In "Single-Cell Protein," (Ed.) R.I. Mateles and S.R. Tannenbaum, p. 91. The M.I.T. Press, Cambridge, MA.
Clifford, A.J. and Story, D.L. 1976. Levels of purines in foods and their metabolic effects in rats. J. Nutr. 106: 435.
Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics 11: 1.
Edozien, J.C., Udo, U.U., Young, V.R., and Scrimshaw, N.S. 1970. Effects of high levels of yeast feeding on uric acid metabolism of young men. Nature 228: 180.
Englyst, H., Wiggins, H.S., and Cummings, J.H. 1982. Determination of the nonstarch polysaccharides in plant foods by gas-liquid chromatography of constituent sugars as alditol acetates. Analyst 107: 307.
Goering, H.K. and Van Soest, P.J. 1970. Forage fiber analyses. U.S. Department of Agriculture, Handbook No. 379: 1.
Gutman, A.B. and Yu, T.F. 1965. Uric acid metabolism in normal man and in primary gout. New Engl. J. Med. 273: 252.
Hartwick, R.A. and Brown, P.R. 1976. Evaluation of microparticle chemically bonded reversed-phase packings in the high-pressure liquid chromatographic analyses of nucleosides and their bases. J. Chromatogr. 126: 679.
Herbert, V. and Bertino, J.R. 1967. Folic acid. In "Vitamins, Chemistry, Physiology, Pathology, Methods," Volume VII, 2nd ed., (Ed.) P. Gyorgy and W.N. Pearson, p. 243, Academic Press, New York.
Kihlberg, R. 1972. The microbe as a source of food. Ann. Rev. Microbiol. 26: 427.
Litchfield, J.H. 1983. Single cell proteins. Science 219: 740.
Marshak, A. and Vogel, H.J. 1951. Microdetermination of purines and pyrimidines in biological materials. J. Biol. Chem. 189: 597.
Martini, A.E.V., Miller, M.W., and Martini, A. 1979. Amino acid composition of whole cells of different yeasts. J. Agric. Food Chem. 27: 982.
McCance, R.M. and Widdowson, E.M. 1978. "The Composition of Foods," (Ed.) A.A. Paul and D.A.T. Southgate. Med. Res. Council Spec. Report No. 297, 4th ed., p. 273. H.M. Stationary Office, London.
Mongeau, R. and Brassard, R. 1982. Determination of neutral detergent fiber in breakfast cereals: pentose, hemicellulose, cellulose and lignin content. J. Food Sci. 47: 550.
Oeryzlo, M.A. 1965. Hyperuricemia induced by high fat diets and starvation. Arthritis Rheum. 8: 799.
Orr, M.L. 1969. Pantothenic acid, vitamin B6 and vitamin B12 in foods. Home Econ. Res. Report 36, USDA, Washington, DC.
PAG. 1975. PAG ad hoc working group on clinical evaluation and acceptable nucleic acid levels of SCP for human consumption. Protein-Calorie Advisory Group of the United Nations System (PAG) Bulletin Volume V(3): 17.
Perloff, B.P. and Butrum, R.R. 1977. Folacin in selected foods. J. Am. Diet. Assoc. 70: 161.
Protein Update 78. 1978. Part II, Guide to protein products and companies. Food Processing July: 44.
Salo, M.L. 1977. The carbohydrate composition and solubility of Pekilo Protein and two yeasts. Acta Agric. Scand. 27: 77.
Sarwar, G., Christensen, D.A., Finlayson, A.J., Friedman, M., Hackler, L.R., Mackenzie, S.L., Pellett, P.L., and Tkachuk, R. 1983. Inter- and intra-laboratory variation in amino acid analysis of food proteins. J. Food Sci. 48: 526.
Scott, R.W. 1979. Colorimetric determination of hexuronic acids in plant materials. Anal. Chem. 51: 936.
Tiemeyer, W., Erbersdobler, H., and Giesecke, D. 1981. Characterization of the nucleic acid fraction and amino acid patterns in single cell proteins as a potential food source. Z. Lebensm. Unters. Forsch. 173: 301.
Tkachuk, R. 1969. Nitrogen-to-protein conversion factors for cereals and oilseed meals. Cereal Chem. 46: 419.
Waldron, C. and Lacroute, F. 1975. Effect of growth rate on the amounts of ribosomal and transfer ribonucleic acids in yeast. J. Bacteriol. 122: 855.
Waslien, C.I., Calloway, D.H., and Margen, S. 1968. Uric acid production of men fed graded amounts of egg protein and yeast nucleic acid. Am. J. Clin. Nutr. 21: 892.
Wright, L.D. and Skeggs, H.R. 1944. Determination of biotin with Lactobacillus arabinosus. Proc. Soc. Exp. Biol. Med. 56: 95.
Zook, E.G., MacArthur, M.J., and Toepfer, E.W. 1956. Pantothenic acid in foods. USDA Handbook, 97, USDA, Washington, DC.

TORTILLA PROCESSING EFFECTS ON AFLATOXIN . . .

Coomes, T.J., Crowther, P.C., Feuell, A.J., and Francis, B.J. 1966. Experimental detoxification of groundnut meals containing aflatoxin. Nature 209: 406.
Felton, J.S., Healey, S., Stuerner, D., Berry, C., Timourian, H., Hatch, F.T., Morris, M., and Bjeldanes, L.F. 1981. Mutagens from the cooking of food. I. Improved extraction and characterization of mutagens from cooked ground beef. Mut. Res. 88: 33.
Martinez, R.R. 1979. Las aflatoxinas en las tortillas. Veterinaria Mexico 10: 37.
Parker, W.A. and Melnick, D. 1966. Absence of aflatoxin from refined vegetable oils. J. Am. Oil Chem. Soc. 43: 645.
Price, R.L. and Sanchez, A. 1979. Aflatoxin destruction during tortilla manufacture. Unpublished results. University of Arizona.
Shotwell, O.L., Goulden, M.L., and Hesseltine, C.W. 1974. Aflatoxin: distribution in contaminated corn. Cereal Chem. 51: 492.
Sosulski, F., Krygier, K., and Hagge, L. 1982. Free, esterified and insoluble-bound phenolic acids. 3. Composition of phenolic acids in cereal and potato flours. J. Agric. Food Chem. 30: 337.
Spingarn, N.E., Garvie-Gould, C.T., and Slocum, L.A. 1983. Formation of mutagens in sugar-amino acid model systems. J. Agric. Food Chem. 31: 301.
Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics," McGraw-Hill Book Company, Inc., New York, NY.
Stoloff, L. 1979. The three eras of fungal toxin research. J. Am. Oil Chem. Soc. 56: 784.
Sugimura, T. and Nagao, M. 1979. Mutagenic factors in cooked foods. CRC Critical Reviews in Toxicology, p. 189.
Thean, J.E., Lorenz, D.R., Wilson, D.M., Rodgers, K., and Gueldner, R.C. 1980. Extraction, cleanup and quantitative determination of aflatoxins in corn. J. Assoc. Off. Anal. Chem. 63: 631.
Ulloa-Sosa, M. and Schroeder, H.W. 1969. Note on aflatoxin decomposition in the process of making tortillas from corn. Cereal Chem. 46: 397.
Wong, J.J. and Hsieh, D.P.H. 1976. Mutagenicity of aflatoxins related to their metabolism and carcinogenic potential. Proc. Natl. Acad. Sci. USA 73: 2241.

Effects of Oxygen Absorber and Temperature on ω3 Polyunsaturated Fatty Acids of Sardine Oil during Storage

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─────────── ABSTRACT ───────────

To prevent loss of ω3 polyunsaturated fatty acids over long-term preservation, the effects of temperature and oxygen absorber on the fatty acids of sardine oil stored in air-tight film were studied. The fatty acids of sardine oil and lipids in the diet of experimental animals rapidly decreased over 1 month at 22°C. The amounts in the diet decreased slowly at 2°C; however, no alterations in the oil samples were observed for 6 months. Also, the amounts did not change at −30°C. Significant changes in samples treated with oxygen absorber were not observed under all temperatures during 6 months storage (P > 0.05). These results indicate that treatment with an oxygen absorber and/or freezing can prevent ω3 polyunsaturated fatty acids of fish oil from decreasing during storage.

INTRODUCTION

FISH OIL CONSUMPTION has been linked to reduced rates of atherosclerotic disease in Greenland Eskimos (Dyerberg et al., 1978; Dyerberg and Bang, 1979). Also, the administration of fish, fish oils or fish oil concentrates lowers serum lipids and reduces the response of platelets to aggregating agents in human subjects and experimental animals (Ruiter et al., 1978; Von Lossonczy et al., 1978; Van Gent et al., 1979; Sanders and Roshanai, 1983; Socini et al., 1983). It is believed that these biological effects of fish oils are caused by ω3 polyunsaturated fatty acids (especially, eicosapentaenoic acid) present in fish oils. The fish oils are unstable to oxidation, since they contain large amounts of the polyunsaturated fatty acids. The oxidative degradation of the fatty acids is a major problem resulting in decreased nutritive value and quality of fish oils. To prevent fats and oils from oxidizing and to preserve the freshness and flavor of foods, an oxygen absorber with the packaged system has been developed. It has been reported that the agents suppress the increase of peroxide and acid values of fish oils by removing the oxygen from the container in the packaged system (Saito, 1979; Uchiyama et al., 1980). However, there is little information on the effects of oxygen absorbers on ω3 polyunsaturated fatty acids of fish oils at various temperatures during long-term storage. This study reports the effects of an oxygen absorber and temperature (22°C, 2°C and −30°C) on eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6) contents of sardine oil during 6 months storage.

MATERIALS & METHODS

Preparation and storage of samples

Sardine oil (A.V. 3.42) was obtained from Federation of North Pacific District Purse Seine Fisheries Cooperative Associations of Japan. The sardines (avg wt 80g) were caught by a purse seiner in the Pacific Ocean (the coast of Chosi) on June 3, 1983. The next day, sardine oil was produced from the fish in a plant according to the conventional processing method i.e. cooking (90°C, 20 min), pressing, and oil purification by centrifugation.

Two types of samples were used for this study: an original sardine oil and a powdered diet for experimental animals containing 10% sardine oil. The composition of the diet is shown in Table 1. Ten milliliters of sardine oil and 30g of the diet were placed in 50 mL and 100 mL beakers, respectively. Each sample was inserted in a pouch, 28 cm × 20 cm, of polyvinylidene chloride-coated oriented nylon/polyethylene laminates [KON (22μ)/PE (65μ)] with or without an oxygen absorber (AGELESS, S-200: activated iron oxide packed with an air-permeable packaging material) and an indicator of deoxygenation (AGELESS EYE: a tablet containing thiazine dye), and the pouches were heat sealed immediately. Both oxygen absorber and indicator of deoxygenation were obtained from Mitsubishi Gas Chemical Co., Inc. After it was confirmed by the indicator that oxygen concentration in the test package decreased to 0.1% or less, the package was used for the experiments.

Each type of test package (treated with or without oxygen absorber) was stored at 22°C, 2°C and −30°C for 6 months. After 0, 1, 3 and 6 months, EPA and DHA of the oil in each package of 12 groups (2 samples × 2 types × 3 temperatures) were quantitated by using gas chromatography (GC).

All results are reported as the mean ± standard deviation, and significant differences between experimental and initial groups were determined by Student's t-test (Snedecor and Cochran, 1976).

Measurement of fatty acids

To 0.5g of sardine oil, 50 mg of tricosanoic acid (23:0) as an internal standard and KOH-methanol as a saponifier were added, and the mixture was heated at 90°C for 1 hr. Lipids of the powdered diet (5g) were extracted by the method of Bligh and Dyer (1959). The lipids were saponified in the same manner as the sardine oil. The resulting mixtures of fatty acids were extracted with diethyl ether under a strongly acidic condition, washed with distilled water and concentrated. The isolated fatty acids were transesterified with N,N-dimethylformamide dimethyl acetal at 80°C for 1 hr (Thenot et al., 1972). The fatty acid methyl esters were directly subjected to GC.

GC was carried out on a Shimadzu GC-6A gas chromatograph equipped with a flame ionization detector and a glass column (3 m ×

Table 1—Composition of the powdered diet

Ingredients	Percent
Corn starch	45.2
Casein	20.0
Sucrose	20.0
Sardine oil	10.0
Salt mixture[a]	4.0
Fat soluble vitamin[b] (soybean oil)	0.5
Methionine	0.2
Water soluble vitamin[c]	0.1

[a] Composition in g/kg: $CaHPO_4 \cdot 2H_2O$, 335.6; $K_3C_6H_5O_7 \cdot H_2O$, 237; $CaCO_3$, 163.5; NaCl, 108; K_2HPO_4, 77.36; $MgCO_3$, 40.9; $FeC_6H_5O_7 \cdot 3H_2O$, 16.01; $MnSO_4$, 1.24; $CuSO_4 \cdot 5H_2O$, 0.178; $CoCl_2 \cdot 6H_2O$, 0.089; $K_2Al_2(SO)_4$, 0.089; $ZnCO_3$, 0.044; KI, 0.044; NaF, 0.0009.
[b] Soybean oil (g) contained: retinyl acetate, 1400 IU; cholecalciferol, 400 IU; α-tocopheryl acetate, 1.40 mg; menadione, 0.60 mg.
[c] Composition in g/kg: choline chloride, 659; inositol, 263; nicotinic acid, 19.8; para-amino benzoic acid, 19.8; thiamin, hydrochloride, 5.2; riboflavin, 5.2; pyridoxine-HCl, 5.2; folic acid, 2.6; biotin, 0.13; cyanocobalamin, 0.04.

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3 mm) packed with Unisole 3000 on 80-100 Uniport C (Gasukuro Kogyo Inc.). The temperatures of the column, injection port and ionization chamber were kept at 235°C, 250°C and 250°C, respectively. Nitrogen was used as the carrier gas at the flow rate of 30 mL/min. This procedure separated the methyl esters of fatty acids from 14:0 to 22:6 within 30 min. Areas under peaks were measured by an electronic digital integrator (Shimadzu C-R1A chromatopac). Components were identified by comparison of the GC retention times with known methyl ester standards. The fatty acids were shown as mg of 23:0. The results of fatty acid analysis by this method were similar to those by the conventional method using BF_3-methanol as an esterification reagent and DEGS as a liquid phase of column packing material of GC. However, this method was more rapid and easy than the conventional one.

RESULTS & DISCUSSION

Fatty acid composition of sardine oil and lipids of the diet

Initial fatty acid composition of sardine oil and lipids of the powdered diet is shown in Table 2. EPA (20:5) and DHA (22:6) contents of sardine oil were about 150 and 52 mg/g as 23:0, respectively. The composition indicates that sardine oil used for these experiments is ordinary. The pattern of fatty acid composition of the diet lipids was similar to that of sardine oil, except that lipoleic acid (18:2) percentages of the lipids were higher than those of sardine oil. Most of linoleic acid of the lipids comes from the 0.5% soybean oil added in the diet.

Effects of temperature on EPA and DHA during storage

Effects of temperature on EPA and DHA of sardine oil and lipids of the diet during storage (without oxygen absorber treatments) are shown in Fig. 1 and 2. Both EPA and DHA of these samples decreased (sardine oil, $P < 0.05$;

the diet, $P < 0.01$) rapidly within 1 month at 22°C. However, after that the contents did not change. The results showed that the packaging of sardine oil using the airtight film prevented the EPA and DHA from decreasing more than the fixed levels. Also, EPA and DHA of the lipids in the diet were reduced to a greater extent than those of sardine oil. Both EPA and DHA of the lipids in the diet decreased slowly at 2°C ($P < 0.05$), but the amounts in the sardine oil did not change ($P > 0.05$). The difference between the results of sardine oil and the diet may be due to the area of contacting surface with oxygen and the balance of oxygen and oil contents, but the mechanisms are not clear in this study. No significant alterations of these fatty acid in the samples were observed at -30°C ($P > 0.05$).

Effects of oxygen absorber on EPA and DHA during storage

EPA and DHA of the lipids in the diet treated with oxygen absorber during storage are shown in Fig. 3. Significant changes of EPA and DHA of the lipids were not observed under all temperature conditions during 6 months ($P > 0.05$). Also, the same results were obtained with experiments using sardine oil. These results indicated that the oxygen absorber has suppressive effects on the loss of EPA and DHA in sardine oil and lipids in the diet during storage.

It is well-known that the packed system using an oxygen absorber and air-tight film has preventive effects on the formation of peroxides, the outbreak of microorganisms and insects, and the discoloration of foods (Saito, 1979). Moreover, the results in this study indicate that the system is very useful for a long-term preservation of foods and animal diets containing fish oil from the viewpoint of preventing the loss of ω3 polyunsaturated fatty acids.

REFERENCES

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911.

Dyerberg, J. and Bang, H.O. 1979. Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. Lancet ii: 433.

Dyerberg, J., Bang, H.O., Stofferson, E., Moncada, S., and Vane, J.R. 1978. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis. Lancet ii: 117.

Ruiter, A., Jongbloed, A.W., Van Gent, C.M., Danse, L.H.J.C., and Metz, S.H.M. 1978. The influence of dietary mackerel oil on the condition of organs and on blood lipid composition in the young growing pig. Am. J. Clin. Nutr. 31: 2159.

Saito, M. 1979. Food quality preservation by means of free-oxygen absorber. Food Industr. 22(10): 65.

Sanders, T.A.B. and Roshanai, F. 1983. The influence of different types of ω3 polyunsaturated fatty acids on blood lipids and platelet function in healthy volunteers. Clin. Sci. 64: 91.

Snedecor, G.W. and Cochran, W.G. 1967. "Statistical Methods," 6th ed. Iowa State University Press, Ames, IA.

Socini, A., Galli, C., Colombo, C., and Tremoli, E. 1983. Fish oil administration as a supplement to a corn oil containing diet affects arterial prostacyclin production more than platelet thromboxane formation in the rat. Prostaglandins 25: 693.

Table 2—Fatty acid composition of sardine oil and lipids of the diet

Fatty acid	Sardine oil (mg/g as 23:0)	Diet (mg/10g as 23:0)
14:0	79.7 ± 6.7[a]	72.5 ± 2.5
16:0	187.7 ± 9.8	180.7 ± 7.4
16:1	102.4 ± 6.5	97.3 ± 4.1
18:0	53.1 ± 2.1	52.6 ± 1.4
18:1	145.4 ± 6.1	152.8 ± 3.6
18:2	16.3 ± 4.2	47.9 ± 3.5
20:0	23.9 ± 1.0	21.6 ± 1.0
20:1	24.0 ± 2.2	22.0 ± 2.0
20:4	9.1 ± 0.4	9.0 ± 0.4
20:5	150.3 ± 5.5	146.0 ± 5.8
22:6	52.0 ± 3.2	48.7 ± 2.7

[a] Values represent the mean ± standard deviation for triplicate analyses.

Fig. 1—Effects of temperature on EPA (●) and DHA (▲) contents of sardine oil during storage. Each point represents the mean ± standard deviation for triplicate analyses.
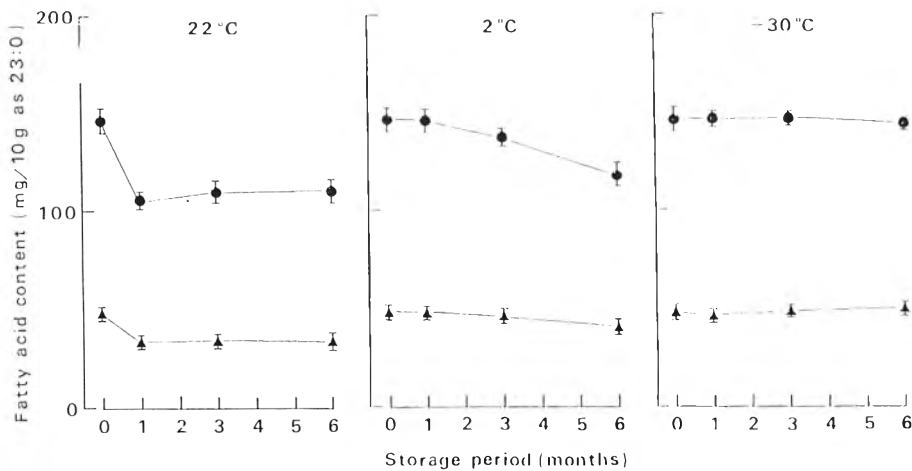
Fig. 2—Effects of temperature on EPA (•) and DHA (▲) contents of lipids of the diet during storage. Each point represents the mean ± standard deviation for triplicate analyses.
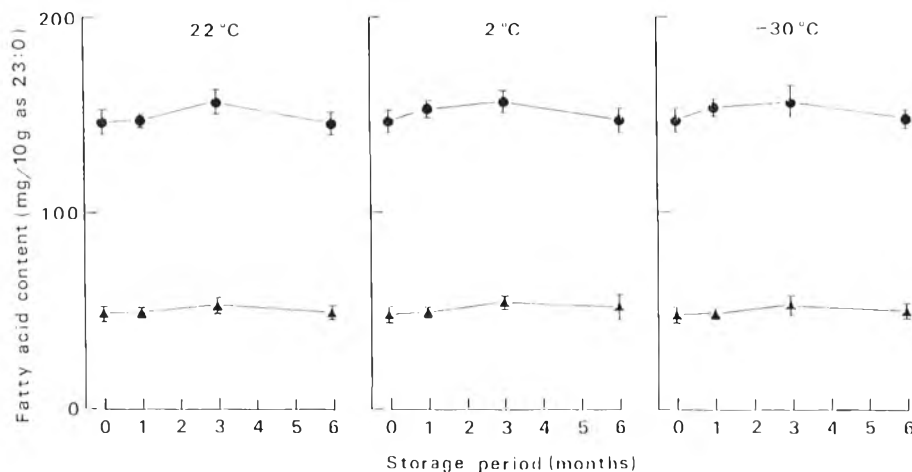
Fig. 3—Protective effect of oxygen absorber on the loss of EPA (•) and DHA (▲) contents of the diet lipids during storage. Each point represents the mean ± standard deviation for triplicate analyses.

Thenot, J-P., Horning, E.C., Stafford, M., and Horning, M.G. 1972. Fatty acid esterification with N,N-dimethylformamide dialkyl acetals for GC analysis. Anal. Letters 5: 217.

Uchiyama, H., Ehira, S., Kakuda, K., Uchiyama, T., Nakamura, H., and Uchida, Y. 1980. A new method for long period preservation of semi-dried fish and baked eel, "Shirayaki". Bull. Tokai Reg. Fish. Res. Lab. No. 102: 31.

Van Gent, C.M., Luten, J.B., Bronsgeest-Schoute, H.C., and Ruiter, A. 1979. Effect, on serum lipid levels of ω-3 fatty acids, of ingesting fish-oil concentrate. Lancet ii: 1249.

Von Lossonczy, T.O., Ruiter, A., Bronsgeest-Schoute, H.C., Van Gent, C.M., and Hermus, R.J.J. 1978. The effect of a fish diet on serum lipids in healthy human subjects. Am. J. Clin. Nutr. 31: 1340.

STABILITY OF GLUTAMIC ACID . . .

Goodban, A.E., Stark, J.B., and Owens, H.S. 1953. Amino acids content of sugar beet processing juices. J. Agr. Food Chem. 1: 261.

Hac, L.R., Long, M.L., and Blish, M.J. 1949. The occurrence of free L-glutamic acid in various foods. Food Technol. 3: 351.

Lin, Y.D., Clydesdale, F.M., and Francis, F.J. 1970. Organic acid profiles in thermally processed spinach puree. J. Food Sci. 35: 641.

Lin, Y.D., Clydesdale, F.M., and Francis, F.J. 1971. Organic acid profiles of thermally processed, stored spinach puree. J. Food Sci. 36: 240.

Mahdi, A.A., Rice, A.C., and Weckel, K.G. 1959. Formation of pyrrolidonecarboxylic acid in processed fruit and vegetable products. J. Agr. Food Chem. 7: 712.

Mahdi, A.A., Rice, A.C., and Weckel, K.G. 1961. Effect of pyrrolidonecarboxylic acid on flavor of processed fruit and vegetable products. J. Agr. Food Chem. 9: 143.

Olcott, H.S. 1944. A method for the determination of glutamic acid in proteins. J. Biol. Chem. 153: 71.

Rice, A.C. and Pederson, C.S. 1954. Chromatographic analysis of organic acids in canned tomato juice, including the identification of pyrrolidonecarboxylic acid. Food Res. 19: 106.

Shallenberger, R.S. and Moyer, J.C. 1958. Relationship between pyrrolidonecarboxylic acid and an off-flavor in beet puree. J. Agr. Food Chem. 6: 604.

Shallenberger, R.S., Palleson, H.R., and Moyer, J.C. 1959. Formation of pyrrolidonecarboxylic acid during the production of beet puree. Food Technol. 13: 92.

Spackman, D.H., Stein, W.H., and Moore, S. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30: 1190.

Wilson, H. and Cannan, R.K. 1937. The glutamic acid-pyrrolidonecarboxylic acid system. J. Biol. Chem. 119: 309.

Rice Bran Stabilization by Extrusion Cooking for Extraction of Edible Oil

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─────────ABSTRACT─────────

An extrusion cooking procedure was developed which produces stable rice bran which shows no significant increase in free fatty acid content for at least 30 – 60 days. In the optimum process, 500 kg/hr of 12 – 13% moisture bran was extruded at 130°C and held 3 min at 97 – 99°C before cooling. Stabilized bran contained 6 – 7% moisture and was in the form of small flakes with 88% larger than 0.7 mm (25 mesh). Energy required to extrude the bran was 0.07 – 0.08 kW-hr/kg bran, and wear on the extrusion surface indicated a life of 500 hr for the cone and 1000 – 2000 hr for other wearing parts.

INTRODUCTION

RICE BRAN, a by-produce of rice milling, is a largely untapped source of edible oil, particularly in developing countries. Depending on milling procedures and hull contamination, rice bran contains 15 – 22% oil, about the same as soybeans (Enochian et al., 1981). Upon milling, the neutral oil is exposed to lipases in the bran, causing its rapid breakdown to free fatty acids (FFA) at an initial rate of at least 5 to 7% of the weight of oil per day (Desikachar, 1974). Since refining losses in the production of edible oil are more than twice the FFA content (Enochian et al., 1981), extraction from rice bran must take place as soon as possible after milling to economically obtain food-grade rice oil. This is not feasible in most developing countries because of lack of integration of milling and extraction facilities, and the oil usually is extracted for lower grade industrial uses or is not separately utilized at all.

Oil deterioration can be prevented by stabilizing the bran (inactivating lipases) immediately after milling. Once the bran is stabilized it can be transported and stored for 30 – 60 days at ambient conditions without appreciable increase in FFA content. Rice bran stabilization methods have been reviewed by Sayre et al. (1982). Heat inactivation of the lipases appears to be the only method with commercial potential. Dry heating or roasting prevents enzyme activity by lowering the bran moisture content to 2 – 3%, but if the bran moisture is subsequently allowed to increase to atmospheric equilibrium of 10 – 13%, lipases frequently become active again (Loeb et al., 1949). Furthermore, this method often subjects the bran to excessively high temperatures. Heating in the presence of moisture is much more effective in permanently denaturing lipases, and this denaturation can be accomplished at milder temperatures (Barber et al., 1974). Steam injection (added moisture) or pressurized heating utilizing the 11 – 13% moisture present in the bran (retained moisture) are two methods used to heat bran in a high moisture environment (Sayre et al., 1982).

Since rice mills in many developing countries have electricity but no source of steam, retained moisture stabiliza-

tion appears to be the most promising process for high moisture heat treatment of bran (Enochian et al., 1981). Two methods of retained-moisture heating which have been tested are rotating sealed drum heaters (Ramanathan et al., 1977) and extrusion cookers (Tribelhorn et al., 1979; Cheigh et al., 1980). In the latter machines, mechanical friction in the extruder heats the bran and extrusion back pressure prevents moisture loss. A preliminary operational and financial feasibility study conducted by Enochian et al. (1981) indicated that stabilization by extrusion cooking would be practical in certain developing countries.

The Brady Crop Cooker has been used extensively for extrusion of soybeans and corn and has proven to be satisfactory and reliable (Tribelhorn et al., 1979; Bressani et al., 1978). Its chief advantages are relatively low capital investment, simplicity of design and operation, low maintenance, and inexpensive operation (Harper, 1979). This machine was tested in a preliminary manner for stabilizing rice bran, and extrusion temperatures of 130 – 140°C were found to prevent lipase activity (Harper et al., 1978). The present study of extrusion cooking was conducted, using a Brady Crop Cooker, to examine in detail the operating conditions necessary to stabilize rice bran and to determine energy consumption, quality of rice oil extracted from stabilized bran, and feed values of both full-fat and defatted stabilized brans. Oil quality and feed values will be reported in later publications.

MATERIALS & METHODS

Pilot plant installation

A pilot plant for extrusion stabilization of rice bran was installed at the Pacific International Rice Mills, Inc. in Woodland, CA. A diagram of the system is shown in Fig. 1. Rice bran was diverted from the bran millstream and delivered within 10 min after milling into the feed hopper of a Brady Crop Cooker, Model 2160, equipped with a 100-hp electric motor (Brady Extruder Corp., Torrance, CA). Moisture addition to the bran was achieved by spraying water from a nozzle into the feed hopper. Extruded hot bran was expelled onto an insulated conveyor belt, where it was held for a specified time period at 97 – 99°C before delivery to a rotating drum air cooler, where countercurrent ambient air was blown over the bran as it was tumbled by flights welded to the inside of the drum. Cooled bran was transported by an elevator conveyor to the bran handling station, for packing in drums or other containers. Bran temperature at this point was 43 – 48°C, and moisture content was about 6%.

After bran feed rate, moisture addition rate to the raw bran, extrusion temperature, and extruded bran holding time prior to cooling were set, the system was allowed to reach steady state before samples and data were collected. Steady state was defined as being achieved when extrusion and cooled bran temperatures each varied by no more than 1°C in a 20-min period. Extrusion temperature was measured by insulated thermocouples taped to the outside of the cup housing, at the extrusion cone.

Experimental design

An initial study was conducted to approximately locate the range of processing conditions required for stabilization. A matrix of processing variables was established with: moisture addition (0 – 4%), bran feed rate (250 – 520 kg/hr), extrusion temperature (90 – 150°C), holding time (0 – 30 min).

Using results obtained from the preliminary study, a second study was carried out with the following ranges of variables: water addition (0 – 3%), bran feed rate (450 – 500 kg/hr), extrusion temperature (120 – 140°C), holding time (0 – 10 min). Experiments at each set of conditions were replicated from two to five times.

Energy requirements

Power required to run the extruder was measured directly by an ammeter connected to the electric drive motor. Total energy use was indicated by a cumulative ammeter. Minor energy consumption by conveyor and cooler motors was not measured.

Sieve analysis

Particle size analysis was carried out on a Rotap device with 10 screens, ranging from 5 mesh (4 mm) to 80 mesh (0.180 mm). The unit was shaken for 5 min. The stability of bran flakes was tested by agitating samples for 30 sec in a tumbling Y-type blender with a spinning agitator bar (Patterson-Kellog Co., East Stroudsburg, PA), after which the samples were sieved again.

Extruder wear

Mechanical wear in the extruder was expected to occur over an extended period of operation, especially around the processor cone at the discharge end (Fig. 2). The extent of wear was determined by comparing measurements of the worn parts to corresponding measurements of new parts. Measurements were made of the inside and outside diameters and bevel angles of the processor cone and the maching bearing cup, of the rotor diameter at several points, and of the heights of several turns of the flight.

Laboratory analysis

Immediately after collection, bran samples were packed in dry ice and stored until analyses were completed. Sample moisture contents were determined by drying 6 hr at 110°C. Peroxidase activity was assayed by the method of Vetter et al. (1958). Residual peroxidase activity in processed rice bran was calculated on a dry weight basis as percentage of activity in raw bran sampled during the same processing run. Peroxidase inactivation was used to give a rapid indication of the effects of processing conditions, for it is considered to be one of the more heat stable plant enzymes (Barber et al., 1974), and lipase inactivation was assumed to precede if not parallel that of peroxidase.

Lipase activity was determined by measuring the increase in FFA content of oil extracted before and after bran samples were incubated in sealed containers for 96 hr at 32°C. Processed bran samples were adjusted to 11% moisture (similar to raw bran) prior to incubation. Lipids were extracted from 10g samples with hexane in a Soxhlet extractor for at least 6 hr and recovered in a total volume of 100 mL. FFA content was determined by a modification of AACC method 02-01 (AACC, 1976) by removing the solvent from 10 mL of extract, dispersing the lipid residue in 75 mL of isopropyl alcohol followed by 75 mL of 0.04% phenolphthalein in 95% ethanol (neutralized with 0.2N KOH to a faint pink color), and titrating duplicate or triplicate 50-mL volumes with standard 0.016N KOH (prepared daily from a 0.2N stock solution) to the first appearance of a peach tint. A blank consisting of 50 mL of a 1:1 mixture of isopropyl alcohol and neutralized 0.04% phenolphthalein in 95% ethanol was also titrated. FFA content was calculated as oleic acid and expressed as weight percent of the total lipid. The total lipid content was measured gravimetrically after desolventizing 50 mL of the hexane extract and drying the residual oil at 110°C for 15 min. Percentage of oil in the bran was expressed on a dry weight basis.

The long term stability of extrusion cooked bran was tested by storing duplicate 1 kg samples of processed or raw bran in clean cotton bags at 32°C and 85% RH for 28 days. The effect of contamination by small quantities of raw bran, as might be found in reused gunny bags, was also tested in the above storage conditions by inoculating processed bran with 0.1% of either fresh raw bran or aged raw bran from a storage warehouse. The raw bran was either shaken in the cotton bag prior to filling with processed bran or mixed with the processed bran prior to filling the bag. All samples were analyzed for increase in FFA content periodically throughout the storage period.

Total aerobic microbiological plate counts were run according to AACC Method 42-11 (AACC, 1976) with these modifications: microorganisms were determined using 0.1% bacto-peptone (Difco), plated on Plate Count Agar (Difco) plate poured 3 days previously and incubated at 30°C for 1 – 3 days, until colonies became visible.

RESULTS & DISCUSSION

Stabilization tests

The influences of raw bran moisture level and extrusion temperature on peroxidase inactivation when bran was cooled immediately after extrusion are shown in Fig. 3, which includes data from both preliminary and final sets of experiments. Considering that less than 3% residual peroxidase activity was evidence of almost complete lipase inactivation, bran was stabilized at 130°C for all moisture levels and at 120°C except at an initial moisture level of 10.5%. Holding the extruded bran at high temperature before cooling appeared to improve stabilization. In another test where bran was held 3 min at 97 – 99°C before cooling, residual peroxidase was 2.3% or below at 120°C extrusion temperature and 0.5% or less at 130°C, at all moisture levels.

Although the peroxidase results were useful for day to day indicators of bran stabilization, increase in FFA in stored bran was the ultimate criterion for evaluating stability. Increases in FFA from initial levels for bran obtained from both sets of experiments and stored 96 hr at 32°C are shown in Fig. 4 as a function of the processing variables of raw bran moisture, extrusion temperature, and holding time.

Rice bran can be stabilized at an extrusion temperature of 105°C, but only at high moisture (13.4%). An extrusion temperature of 120°C appeared to stabilize rice bran under most moisture conditions (10.5%, 13.4%), and holding time after extrusion did not appear to affect lipase activity. Inconsistencies are probably due to experimental error in FFA determinations. When bran was processed at 130°C,
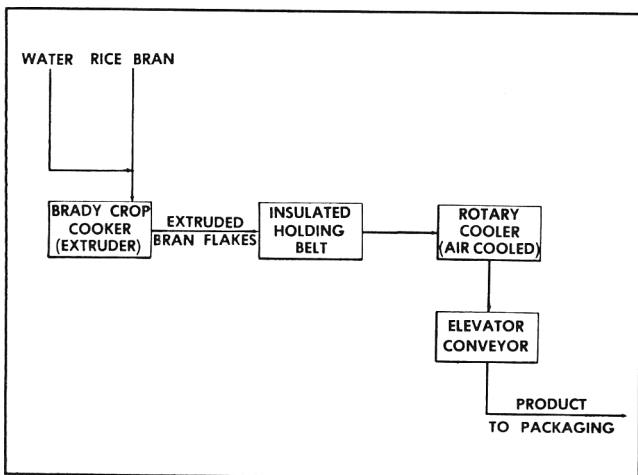
Fig. 1—Schematic of rice bran stabilization system.
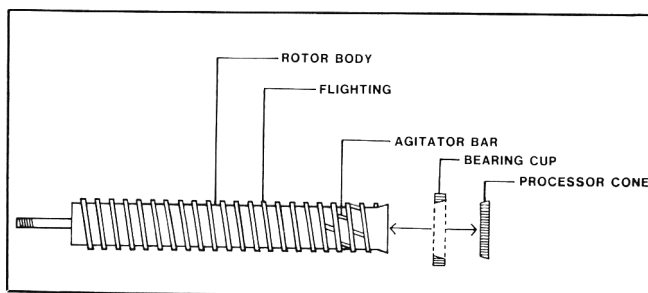
Fig. 2—Rotor and extrusion section.

the increase in FFA content was less than 1% after the four day storage period in all samples. Thus, no addition of water above the level found in raw bran was necessary, and extrusion at temperatures above 130°C is not necessary.

Particle size

Since the ultimate objective of rice bran stabilization is extraction of oil, the particle size distribution of the stabilized bran is of great interest. It is difficult for solvent to percolate through a bed of fine bran to extract the oil. Channeling can occur, leading to uneven and incomplete oil extraction. Raw bran is too fine for extraction in many systems, but extrusion agglomerates the bran particles and forms small flakes. Table 1 shows partial fine particle analyses for raw rice bran and bran extruded at different temperatures. A separate run was made to prepare samples, and the experimental conditions were slightly different than for the stabilization tests. Raw bran contained about 85% fines (defined as <0.7 mm). All extrusion treatments increased flake size, and the least fines were produced at low processing temperatures and high raw bran moisture levels.

Since mechanical handling prior to extraction could reduce particle size, extruded bran samples were subjected to 30 sec severe agitation. There was usually considerable breakage of flakes, as shown in Table 1. The effect of agitation was much less on extruded bran prepared from high moisture raw bran (12.4 and 14.4%) than on extruded bran for 10.5% moisture raw bran. Increased moisture in raw bran appeared to harden the flakes, making them more resistant to breakage.

Energy requirements

Directly measured energy consumption for the extruder alone was consistently in the range 0.07 – 0.09 kW-hr/kg bran (Table 2) for processing rates of 450 kg/hr or higher and increased both with increasing extrusion temperature and with increased moisture. Energy consumption was 14 – 16% greater for an extrusion temperature of 140°C, compared to 130°C.

When approximately 3% water was added to the bran to increase moisture levels to 13.4%, the bran feed rate could not be closely controlled, perhaps due to uneven moisture distribution. Processing rates often decreased from the usual 450 – 500 kg/hr to 400 kg/hr or lower, and energy consumption increased to 0.10 kW-hr/kg bran or higher. This unacceptable energy consumption level obviated advantages of higher moisture bran. In tropical countries, however, where bran moisture levels might equilibrate natur-
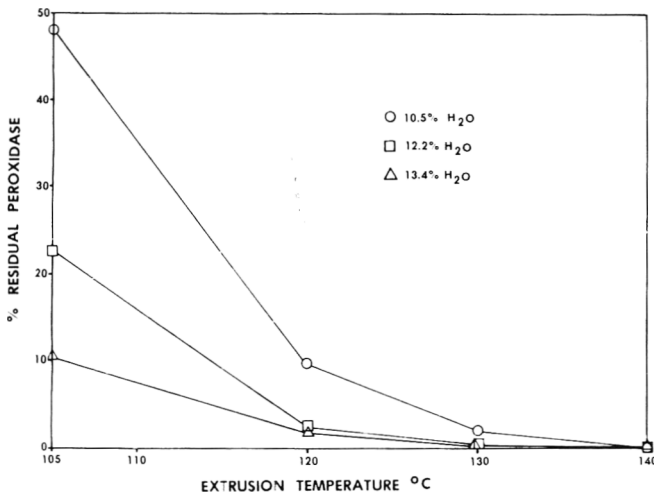
Fig. 3–Residual peroxidase activity in bran extruded at various temperatures and raw bran moisture levels, zero hold time.
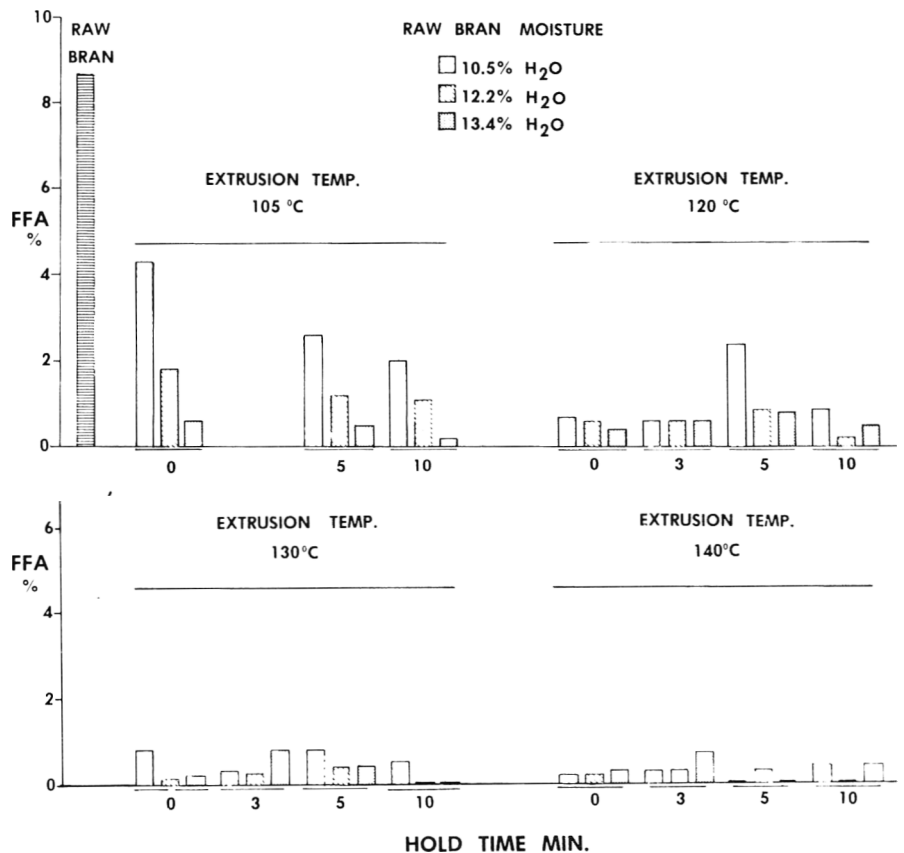
Fig. 4–Free fatty acid increase in rice bran adjusted to 11% moisture, after 96 hr storage at 32°C as influenced by raw bran moisture content, extrusion temperature, and holding time at 97 – 99°C.

ally at 13% or higher, feed rates might not be affected in the same way, eliminating the disadvantage.

Optimal stabilization conditions

Based on the above observations, and allowing for a safety margin, optimal conditions for stabilization were considered to be 12-13% initial bran moisture, throughput of 450-500 kg/hr, extrusion temperature of 130°C, and post-extrusion holding time of 3 min at 97-99°C.

Storage stability

Contamination of processed bran with fresh raw bran or warehouse raw bran had no effect on stored bran characteristics. The long term storage of raw and stabilized brans are compared in Table 3. After 28 days storage at 32°C and 85% RH, the FFA content of stabilized brans, whether contaminated or not, had increased only 1%, while raw bran had increased 47% to a total of 51%. The stabilization process decreased the microbial count to very low levels which did not increase during storage.

A long-term storage test was also conducted over a period of 4.5 months in which raw bran was held at −23° and 32°C and stabilized bran was held at 32°C (Fig. 5). Neither the stabilized bran nor raw bran stored at −23°C showed an increase in FFA content during the entire period. However, the FFA content of raw bran at 32°C increased to over 80%.

Extruder wear

To be successful in continuous commercial operation, the extruder must not wear excessively, or processing conditions can not be kept constant. Measurements of extruder wear were made after 360 hr total running time. The processor cone (entrance diameter 141.0 mm, exit diameter 157.0 mm, taper angle 18.3°), the bearing cup (entrance diameter 140.2 mm, exit diameter 156.5 mm taper angle 18.7°), and the last 3-4 turns of the rotor flights (ht. 7.6 mm) (Fig. 2) all showed some signs of wear. The processor cone decreased in diameter by 1.7 mm at the exit edge and by 3.7 mm at the entrance edge, resulting in an increase of 2.5° in the taper angle. The bearing cup diameter increased 0.1-0.3 mm with only a 0.2° increase of taper angle. The diameter of the last three rotor flights had worn down by 0.2 mm, and the rotor body diameter near the exit end was decreased by 0.2-0.5 mm. In order to prevent steam blow-back, eleven agitator bars were welded

Fig. 5—Free fatty acid increase in raw and stabilized brans (130°C, 3 min hold, 2% added moisture) during a 135 day storage period at −23° or 32°C.

between the flights starting at the exit end. Eight were spaced at 90° intervals and the remaining three at 120° intervals. These bars showed considerable wear but could easily be built up or replaced.

The wear in no way impaired the efficiency of the extruder. It was estimated from previous experience that the cone might have to be replaced after about 500 hr, but other parts mentioned above could operate for at least 1000-2000 hr before replacement, depending upon contamination of the bran with hulls or other extraneous materials.

CONCLUSIONS

ALTHOUGH rice bran was stabilized under some conditions at 120°C, stabilization was always adequate at an extrusion temperature of 130°C. Energy consumption tended to rise only slightly as extrusion temperature increased from 120°C to 130°C, but increased by almost 15% when the temperature was raised from 130°C to 140°C.

It was visually apparent that flake sizes were larger at low temperatures (120°C) but that these flakes were fragile and broke up with any agitation. Extrusion temperatures higher than 130°C resulted in a larger proportion of fines both before and after agitation. Increased moisture content of the raw bran appeared to produce flakes more resistant to breakage.

A consistent temperature of 120°C was usually suitable for stabilization, but since processing control might not

Table 1—Rice bran fines, % < 0.7 mm (25 mesh)

Bran moisture %	Extrusion temperature			
	None	130°C	140°C	150°C
		As extruded		
10.4	85	12	19	22
12.4		7	16	20
14.4		8	8	9
		After agitation		
10.4		26	39	43
12.4		35	34	34
14.4		24	24	27

Table 2—Energy requirement for extrusion at processing rates of > 425 kg/hr

Bran Moisture %	Energy requirement kW-h/kg bran		
	Extrusion temperature		
	120°C	130°C	140°C
10.5	0.066	0.071	0.080
12.2	0.076	0.076	0.088
13.4	0.077	0.080	0.092

Table 3—Storage stability of bran[a]

	Raw bran		Stabilized bran	
	Initial	28 Days	Initial	28 Days
FFA %	4	51	3	4
H_2O %	11	12	7	11
Bacteria X 10^2/g	82000	7400	9	4

[a] 1 kg bran samples stored in cloth bags at 32°C, 85% RH
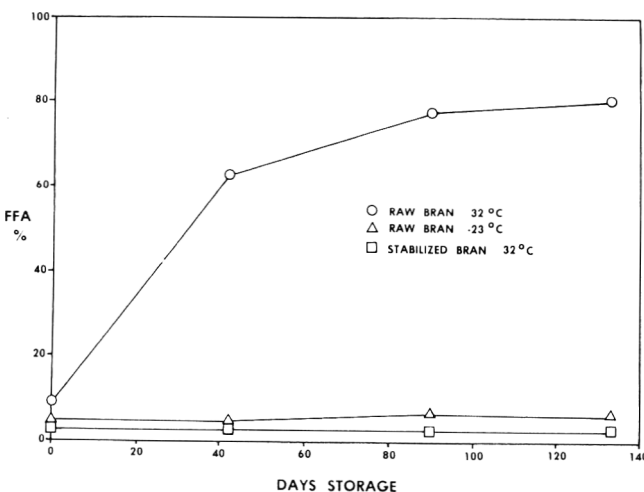
Minerals in Selected Variety Breads Commercially Produced in Four Major U.S. Cities

GUR RANHOTRA, JANETTE GELROTH, FRANCES NOVAK, and RUTH MATTHEWS

─────────────── ABSTRACT ───────────────

Nine mineral elements (Ca. P, Mg, Fe, Zn, Cu, Mn, Na and K) were determined in 10 types, up to four brands of each type, of variety breads purchased from four major U.S. cities. Calcium content of test breads varied appreciably among cities and among brands within a city. In contrast, such variations in the content of Mg, Zn, Cu, and Mn in breads were minimal. Phosphorus was the most abundant mineral in all products. Iron content averaged the highest (3.76 mg/100g) in pumpernickel bread. Sodium in test breads except tortillas averaged between 474 to 734 mg/100g. Four slices of breads tested provide appreciable amounts of various minerals towards our daily need.

INTRODUCTION

OVER THE LAST SEVERAL YEARS, the consumption of variety breads, loosely defined to include breads other than white pan bread, in the United States has been increasing steadily (Ranhotra and Winterringer, 1981). Product quality and nutritional considerations/perceptions appear to have influenced this trend. Studies were recently initiated to widen the base on which nutritional perceptions can be substantiated and documented. This encompassed developing detailed compositional information on ten types of popular variety breads (up to four brands of each type) which were obtained from four major U.S. cities. Earlier we reported (Ranhotra et al., 1984a) on the proximate components in these products. This paper presents information on selected minerals in the same products.

MATERIALS & METHODS

Products and processing

All pertinent information on the 129 products (Table 1) tested and the processing involved was detailed earlier (Ranhotra et al., 1984a). Additional information showing which of the products tested were enriched products is now included in Table 1. Enrichment adds three B vitamins and iron. Calcium, an optional ingredient, may also be included in the enrichment.

Analytical

Triplicates of each sample were analyzed for each of the minerals reported. Total phosphorus in the test products was determined colorimetrically using the standard AACC method (1977). Sodium and potassium were determined by flame emission and the other seven minerals by atomic absorption spectrophotometry using an IL (Allied Analytical Systems, Andover, MA) model 251 spectrophotometer. Samples for Ca and Mg were dry ashed (560°C; 16 hr) and were then analyzed following AOAC (1980) method 2.109 – 2.113. Samples for the other minerals were wet ashed by digestion of a 1-g sample in 30 mL nitric acid, boiling until about 10 mL of solution remained, addition of 5 mL perchloric acid, and heating until evolution of dense white fumes had occurred. The National Bureau of Standards (NBS) wheat flour was analyzed for contained minerals to verify the validity of the analytical methods; analyzed

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values approached or only slightly exceeded the certified values for the seven minerals contained in NBS flour. Averages and standard deviations were calculated for values among cities and within a city.

RESULTS & DISCUSSION

WHOLEWHEAT BREAD and tortillas are traditionally not enriched. For this reason, only the unenriched brands of these two products were selected for analysis (Table 1). In contrast, the selected brands of some (Chicago products) or all (other cities) of other products were enriched. Since all Chicago brands were analyzed separately, enrichment of all was not sought. For each of the other three cities, all brands of a product were composited for analysis and here it was ensured that all products were enriched in preference to not being enriched.

Major minerals

Table 2 lists the contents of four major minerals — calcium, phosphorus, magnesium, and iron — in the test products. These values represent averages of up to four determinations (Chicago) or the grand average of average values for each of the four cities (all-cities). For most products, the calcium content was low. This implies that calcium was not a component of enrichment. Calcium values also varied substantially both within a city (Chicago) and between all cities. Tortillas averaged the highest in calcium content. This likely resulted from the use of lime in processing of the tortillas.

Phosphorus was the most abundant major mineral in the test products, and the amounts in tortillas and wholewheat breads were quite substantive. Some brand to brand differences were noted in the content of phosphorus but they were less pronounced than the differences observed for calcium. Brand to brand differences were also noted in the content of magnesium. However, they were in keeping with the range of values reported earlier (Ranhotra et al., 1984b) for these bread types in a related study. That study

Table 1—Bread types and number of brands tested

Bread type	Cities[a,b]			
	Los Angeles	Dallas	Washington	Chicago
Pumpernickel	4 (4)	3 (3)	2 (2)	4 (1)
Raisin	4 (4)	3 (3)	4 (4)	4 (3)
Oatmeal	2 (2)	1 (1)	1 (1)	3 (3)
Wholewheat (100%)	4 (0)	3 (0)	4 (0)	4 (0)
Cracked Wheat	4 (4)	4 (4)	4 (4)	4 (3)
Mixed Grain	4 (4)	4 (4)	3 (3)	4 (1)
Italian	2 (2)	2 (2)	2 (2)	4 (1)
French	3 (3)	4 (4)	3 (3)	4 (1)
Pita (White)	4 (4)	2 (2)	1 (1)	3 (2)
Tortillas (corn)	3 (0)	4 (0)	3 (0)	4 (0)

[a] For cities other than Chicago, all brands of a product were composited for analysis (equal dry matter basis); for Chicago, the brands were analyzed separately. For the Chicago products, at least three loaves/brand were purchased. Since all brands of a product from each of the other three cities were composited, only one loaf/brand was purchased from these three cities.
[b] Numbers within parenthesis show enriched products

examined just one brand of the test products obtained from four different U.S. cities.

The content of iron averaged (all-cities) the lowest in tortillas and appreciably higher in a number of less-refined bread products such as pumpernickel, cracked wheat, mixed grain, and wholewheat breads. This is due, in part, to the fact that iron, like most other minerals, tends to be higher in the nonendospermic fractions of the grain than in the white endosperm. Some differences were noted in the content of iron between Chicago brands and all-cities brands. These differences would narrow if unenriched brands (Table 1) are eliminated from consideration, including the two Italian breads from Washington which, contrary to the product labels, appear to be unenriched. The conclusion on Italian breads was based on analyzed values not only for iron but also for the three enrichment vitamins.

Trace minerals

The all-cities average values for zinc, copper, and manganese in test breads differed little from the average values for the corresponding breads from Chicago (Table 3) or from values reported for such products earlier (Ranhotra

et al., 1984b). This reflects favorably on the meaningfulness of the compositional data, as are being developed, for use in nutritional studies and practices. Table 3 also shows that certain less-refined bread products would contribute substantially more of the trace minerals in our diet than would the refined products.

Electrolytes

Pumpernickel and Italian breads averaged the highest in sodium content (Table 4). As found earlier (Ranhotra et al., 1984b), brand to brand differences in the content of sodium were also most pronounced for these two bread types. The content of sodium in oatmeal, mixed grain, and Italian breads was also higher this time than reported earlier. The reverse was true for pita breads. While tortillas were earlier found to be virtually free of sodium (only 2 mg/100 g), this time they contained a modest amount. This resulted from one Chicago brand, and one or more brands from other cities, containing added salt. City to city and brand to brand differences in the content of potassium in test products were of a smaller magnitude as compared to sodium. Certain bread types tended to be a more substantive source of potassium in the diet than other bread types (Table 4).

Table 2—Major minerals in breads (mg/100g of product on as-purchased basis)

| Mineral | Bread | City | | | | All cities[b] | |
		Los Angeles	Dallas	Washington	Chicago[a]		
Calcium	Pumpernickel	53	80	55	36 ± 22	56	± 18
	Raisin	66	75	77	52 ± 14	68	± 12
	Oatmeal	34	78	81	57 ± 32	62	± 22
	Wholewheat	69	59	68	84 ± 31	70	± 11
	Cracked Wheat	60	60	58	30 ± 10	52	± 15
	Mixed grain	30	99	138	67 ± 40	84	± 46
	Italian	129	64	93	52 ± 27	84	± 34
	French	73	66	109	53 ± 36	75	± 24
	Pita	49	96	116	67 ± 17	82	± 29
	Tortillas	198	219	199	203 ± 74	205	± 10
Phosphorus	Pumpernickel	128	168	167	191 ± 18	164	± 26
	Raisin	106	106	113	106 ± 15	108	± 4
	Oatmeal	106	129	120	136 ± 6	123	± 13
	Wholewheat	193	223	235	235 ± 16	222	± 20
	Cracked Wheat	142	138	180	151 ± 27	153	± 19
	Mixed grain	156	153	152	202 ± 34	166	± 24
	Italian	115	117	89	112 ± 19	108	± 13
	French	95	110	84	115 ± 18	101	± 14
	Pita	88	98	76	96 ± 6	90	± 10
	Tortillas	287	232	234	323 ±111	269	± 44
Magnesium	Pumpernickel	43	52	56	58 ± 8	52	± 7
	Raisin	28	25	26	29 ± 6	27	± 2
	Oatmeal	32	36	35	45 ± 5	37	± 6
	Wholewheat	81	91	86	92 ± 3	88	± 5
	Cracked Wheat	49	44	59	52 ± 16	51	± 6
	Mixed grain	52	55	54	72 ± 14	58	± 9
	Italian	39	25	24	29 ± 5	29	± 7
	French	28	27	20	31 ± 5	27	± 5
	Pita	25	23	22	26 ± 1	24	± 2
	Tortillas	59	62	66	63 ± 4	63	± 3
Iron	Pumpernickel	5.07	3.76	3.92	2.29 ± 0.40	3.76 ± 1.14	
	Raisin	3.01	4.70	2.54	2.61 ± 0.28	3.22 ± 1.01	
	Oatmeal	3.47	2.88	3.15	2.88 ± 0.14	3.10 ± 0.28	
	Wholewheat	3.65	3.21	2.92	3.33 ± 0.92	3.28 ± 0.30	
	Cracked Wheat	3.84	2.99	3.23	3.58 ± 1.56	3.41 ± 0.38	
	Mixed grain	3.39	4.01	3.42	3.20 ± 0.32	3.51 ± 0.35	
	Italian	3.95	3.37	1.80	2.43 ± 1.30	2.89 ± 0.96	
	French	3.90	3.46	2.97	2.32 ± 1.37	3.16 ± 0.68	
	Pita	3.23	2.32	2.44	1.65 ± 0.46	2.41 ± 0.65	
	Tortillas	1.49	1.21	1.30	1.84 ± 0.24	1.46 ± 0.28	

[a] Values are average of up to four brands ± SD.
[b] Values are grand average of four-city averages ± SD.

Nutrition information

Bread consumption in the U.S. averages about 100g (four slices) per person per day (Gustafson, 1983). Table 5 shows the amount of various minerals four slices would provide towards our daily need expressed as US-RDA (NNC, 1975) or as lower levels of safe intakes (FNB, 1980). It is obvious that certain less-refined products would provide appreciably more of the naturally occurring minerals

Table 3 — Trace minerals in bread (mg/100g of product as-ourchased basis)

Mineral	Bread	City				All cities[b]
		Los Angeles	Dallas	Washington	Chicago[a]	
Zinc	Pumpernickel	1.13	1.95	1.62	1.64 ± 0.20	1.59 ± 0.34
	Raisin	0.74	0.65	0.83	0.83 ± 0.16	0.76 ± 0.09
	Oatmeal	0.79	1.07	0.96	1.16 ± 0.16	1.00 ± 0.16
	Wholewheat	1.74	2.25	1.88	2.13 ± 0.19	2.00 ± 0.23
	Cracked Wheat	1.21	1.14	1.45	1.18 ± 0.26	1.25 ± 0.14
	Mixed grain	1.21	1.27	1.24	1.69 ± 0.40	1.35 ± 0.23
	Italian	1.02	1.03	0.85	0.99 ± 0.14	0.97 ± 0.08
	French	0.80	1.13	0.79	0.94 ± 0.11	0.92 ± 0.16
	Pita	1.00	0.89	0.79	0.85 ± 0.14	0.88 ± 0.09
	Tortillas	1.15	1.05	1.04	1.25 ± 0.03	1.12 ± 0.10
Copper	Pumpernickel	0.23	0.24	0.27	0.31 ± 0.10	0.26 ± 0.04
	Raisin	0.22	0.21	0.22	0.16 ± 0.01	0.20 ± 0.03
	Oatmeal	0.18	0.23	0.25	0.18 ± 0.03	0.21 ± 0.04
	Wholewheat	0.32	0.29	0.30	0.30 ± 0.05	0.30 ± 0.01
	Cracked Wheat	0.20	0.20	0.22	0.21 ± 0.05	0.21 ± 0.01
	Mixed grain	0.26	0.27	0.23	0.29 ± 0.06	0.26 ± 0.03
	Italian	0.26	0.18	0.18	0.18 ± 0.03	0.20 ± 0.04
	French	0.22	0.21	0.25	0.21 ± 0.03	0.22 ± 0.02
	Pita	0.15	0.15	0.15	0.20 ± 0.07	0.16 ± 0.03
	Tortillas	0.15	0.13	0.11	0.13 ± 0.04	0.13 ± 0.02
Manganese	Pumpernickel	1.05	1.31	1.49	1.45 ± 0.33	1.33 ± 0.20
	Raisin	0.54	0.44	0.55	0.58 ± 0.08	0.53 ± 0.06
	Oatmeal	0.74	1.02	1.04	1.06 ± 0.11	0.97 ± 0.15
	Wholewheat	2.03	2.55	2.53	2.68 ± 0.24	2.45 ± 0.29
	Cracked Wheat	1.29	1.07	1.71	1.34 ± 0.51	1.35 ± 0.27
	Mixed grain	1.24	1.27	1.24	1.93 ± 0.66	1.42 ± 0.34
	Italian	0.69	0.49	0.40	0.46 ± 0.05	0.51 ± 0.13
	French	0.55	0.59	0.42	0.54 ± 0.13	0.53 ± 0.07
	Pita	0.48	0.45	0.42	0.46 ± 0.04	0.45 ± 0.03
	Tortillas	0.40	0.44	0.39	0.38 ± 0.04	0.40 ± 0.03

[a] Values are average of up to four brands ± SD.
[b] Values are grand average of four-city averages ± SD.

Table 4 — Electrolytes in breads (mg/100g of product as-purchased basis)

Electrolyte	Bread type	City				All cities[b]
		Los Angeles	Dallas	Washington	Chicago[a]	
Sodium	Pumpernickel	768	617	608	786 ± 181	695 ± 95
	Raisin	459	434	403	389 ± 48	421 ± 31
	Oatmeal	514	664	611	616 ± 121	601 ± 63
	Wholewheat	586	590	493	541 ± 83	553 ± 45
	Cracked Wheat	596	546	477	555 ± 17	544 ± 49
	Mixed grain	733	516	533	571 ± 54	588 ± 99
	Italian	608	627	522	706 ± 180	616 ± 76
	French	628	622	527	619 ± 87	599 ± 48
	Pita	557	569	316	467 ± 36	477 ± 117
	Tortillas	134	21	186	44 ± 79	96 ± 77
Potassium	Pumpernickel	153	203	235	224 ± 32	204 ± 36
	Raisin	252	269	247	250 ± 42	255 ± 10
	Oatmeal	111	125	110	154 ± 27	125 ± 21
	Wholewheat	249	257	280	255 ± 18	260 ± 14
	Cracked Wheat	165	173	196	163 ± 39	174 ± 15
	Mixed grain	159	208	193	232 ± 29	198 ± 31
	Italian	103	99	101	109 ± 5	103 ± 4
	French	115	124	96	126 ± 18	115 ± 14
	Pita	106	126	77	144 ± 30	113 ± 29
	Tortillas	189	159	150	240 ± 64	185 ± 41

[a] Values are average of up to four brands ± SD.
[b] Values are grand average of four-city averages ± SD.

Table 5–Nutrition information per 100g bread[a]

Nutrient	Percent of daily allowance[b]	
	Minimum[c]	Maximum[c]
Calcium	5 (cracked wheat bread)	20 (tortillas)
Phosphorus	9 (pita bread)	27 (tortillas)
Magnesium	6 (pita bread)	22 (wholewheat bread)
Iron	8 (tortillas)	21 (pumpernickel bread)
Zinc	5 (raisin bread)	13 (wholewheat bread)
Copper	7 (tortillas)	15 (wholewheat bread)
Manganese	16 (tortillas)	98 (wholewheat bread)
Sodium	9 (tortillas)	63 (pumpernickel bread)
Potassium	5 (Italian bread)	14 (wholewheat bread)

[a] 100g bread equals four slices.
[b] Expressed as US-RDA or as safe intakes (Mn, Na and K).
[c] Based on lowest and highest values and the products representing them.

than the refined products. This is most striking for the element manganese. Such contribution, however, must be viewed in the context that less-refined products are also usually high in potential inhibitors of mineral absorption (Ranhotra, 1983; Ranhotra and Gelroth, 1983). Also important is the fact that in a mixed diet, small differences in mineral makeup between various bread types are nutritionally inconsequential. In the present study, this is true for most minerals except sodium and iron.

REFERENCES

AACC. 1977. "Approved Methods." American Assoc. Cereal Chemists, Inc., St. Paul, MN.
AOAC. 1980. "Official Methods of Analysis," 13th ed. Association of Official Analytical Chemists, Washington, DC.
FNB. 1980. Recommended Dietary Allowances (9th ed.). National Academy of Sci./National Res. Council, Washington, DC.
Gustafson, N.J. 1983. Consumption and nutritional contributions of wheat foods: Perceptions and Reality. Cereal Foods World 28: 631.
NNC. 1975. Nutrition Labeling — How It Can Work for You. National Nutrition Consortium, Bethesda, MD.
Ranhotra, G.S. 1983. Bioavailability of magnesium in cereal-based foods. Cereal Foods World 28: 349.
Ranhotra, G.S. and Gelroth, J.A. 1983. Zinc bioavailability from cereal-based foods. In "Nutritional Bioavailability of Zinc." Am. Chem. Soc. Washington, DC.
Ranhotra, G.S., Gelroth, J.A., Novak, F.A., Bock, M.A., Winterringer, G.L., and Matthews, R.H. 1984b. Nutritive value of selected variety breads and pastas. J. AM. Diet. Assoc. 84: 322.
Ranhotra, G., Gelroth, J., Novak, F., Bohannon, F., and Matthews, R. 1984a. Proximate components in selected variety breads commercially produced in major U.S. cities. J. Food Sci. 49: 642.
Ranhotra, G.S. and Winterringer, G.L. 1981. Consumption pattern of variety breads in the United States. In "Variety Breads in the United States." American Assoc. Cereal Chemists, Inc. St. Paul, MN.

always be stable, it was decided that an operating temperature of 130°C would provide an adequate safety margin for unanticipated changes in raw bran moisture, extrusion temperature fluctuation, or stabilized bran holding time and would not require escessive energy. Thus, the optimum processing conditions were considered to be raw bran moisture level of 12 – 13%, extrusion temperature of 130°C, processing rates of 500 kg/hr, and a post-extrusion holding time of 3 min. Treatment under such conditions should consistently produce bran which would be stable for 8 weeks or longer.

Some wear of extruder parts was observed after 360 hr running time, but extrusion capability was not diminished. It was estimated that only the cone might require replacement at about 500 hr, but other wearing parts could be run for 1000 – 2000 hr before they would have to be replaced or remachined.

REFERENCES

AACC. 1976. Approved methods of the American Association of Cereal Chemists. AACC, St. Paul, MN.
Barber, S., Camacho, J.M., Cerni, R., Tortosa, E., and Primo, E. 1974. Process for the stabilization of rice bran. 1. Basic research studies. In "Proc. Rice By-Products Utilization Int. Conf." 2: p 49. Valencia, Spain.
Bressani, R., Braham, J.E., Elias, L.G., Cuevas, R., and Molina, M.R. 1978. Protein quality of a whole corn/whole soybean mixture processed by a simple extrusion cooker. J. Food Sci. 43: 1563.
Cheigh, H.S., Kim, C.J., and Kim, D.C. 1980. Development and use of a low-cost extruder for the rice-bran oil stabilizaton at local mill. Presented at Intl. Symp. Recent Advances in Food Sci. Technol. Taipei.
Desikachar, H.S.R. 1974. Status report: Preservation of by-products

of rice milling. In "Proc. Rice By-Products Utilization Int. Conf." 2: p 1. Valencia, Spain.
Enochian, R.V., Saunders, R.M., Schultz, W.G., Beagle, E.C., and Crowley, P.R. 1981. Stabilization of rice bran with extrusion cookers and recovery of edible oil: A preliminary analysis of operations and financial feasibility. Marketing Research Report 1120. ARS, USDA, Washington, DC.
Harper, J.M. 1979. L.E.C. Technology: Where does it fit? In L.E.C. Report No. 7, p. 15. Colorado State Univ., Fort Collins, CO.
Harper, J.M., Cummings, D.A., Kellerby, J.D., Tribelhorn, R.E., Jansen, G.R., and Magor, J.A. 1978. Evaluation of low-cost extrusion cookers for use in L.D.C.s. In L.E.C. Report No. 4, p. 144. Colorado State Univ., Fort Collins, CO.
Loeb, J.R., Morris, N.J., and Dollear, F.G. 1949. Rice bran oil. IV. Storage of the bran as it affects hydrolysis of the oil. J. Am. Oil Chem. Soc. 26: 738.
Ramanathan, P.K., Murty, H.K., and Sen, D.P. 1977. Stabilization of rice bran oil. In Seminar on edible rice bran oil, p. 75. The Solvent Extractors Assoc. Bombay, India.
Sayre, R.N., Saunders, R.M., Enochian, R.V., Schultz, W.G., and Beagle, E.C. 1982. Review of rice bran stabilization systems with emphasis on extrusion cooking. Cereal Foods World 27: 317.
Tribelhorn, R.E., Cummings, D.A., and Kellerby, J.D. 1979. Characteristics of L E C's and manufacturing experiences. In L.E.C. Report No. 7, p. 165. Colorado State Univ., Fort Collins, CO.
Vetter, J.L., Steinberg, M.P., and Nelson, A.I. 1958. Qualitative determination of peroxidase in sweet corn. J. Agr. Food Chem. 6: 39.

Performance Evaluation of Time-Temperature Indicators for Frozen Food Transport

JOHN HENRY WELLS AND R. PAUL SINGH

─────────ABSTRACT─────────

Time-temperature indicators from four different manufacturers were evaluated on the basis of their response to constant and variable storage temperature treatments. Several time-temperature indicators of each type were exposed to constant temperatures of −18°C and +5°C, and a variable temperature treatment which could simulate the conditions encountered in a frozen food transport. The discussion presents the time-temperature response of the indicators tested, the average endpoint response recorded, and comments on the characteristic assets and limitation of the indicators investigated.

INTRODUCTION

A TIME-TEMPERATURE INDICATOR is a device which registers a response according to the combined effect of time and temperature. Well known studies have related the effect of time and temperature to the quality of frozen food (Van Arsdel et al., 1968; Schwimmer and Ingraham, 1955). Because quality is so closely related to this time and temperature interaction, a primary application of the time-temperature indicators could be monitoring the temperature exposure history of frozen foods during distribution. An overview of the types of time-temperature indicators is presented by Byrne (1976) and Farquhar (1977). Schoen (1983) comments on the appropriateness of time-temperature indicators for predicting quality changes in frozen foods.

Several studies on the performance evaluation of time-temperature indicators have been previously undertaken. The most noteworthy among these studies have been reported by Hayakawa and Wong (1974), Kramer and Farquhar (1976), and Arnold and Cook (1977).

All performance evaluation studies thus far have provided useful data about the endpoint measures of time-temperature indicators, but lack any information regarding the kinetic interpretation of the indicator scale readings. In light of the recent interest in shelf life kinetics (Heldman and Lai, 1983; Labuza, 1982; Saguy and Karel, 1980) the information on time-temperature indicator response needs to be revised.

The objective of this investigation was to evaluate the response of time-temperature indicators to a temperature treatment simulating frozen food transport, and to document the responses of various indicators at different storage temperatures.

MATERIALS & METHODS

Types of indicators evaluated

Four manufacturers of time-temperature indicators were contacted and asked to specify a particular model of indicator which was applicable to frozen food transport. All of the manufacturers responded favorably and the specified indicators were obtained. For the purpose of this presentation, the various indicators evaluated shall be referred to as indicator "A", "B", "C", and "D".

Indicators "A", "C" and "D" are designed to be partial-history time-temperature indicators, essentially abuse indicators which

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will yield no response unless a predetermined temperature is exceeded. Because of this, no indicator response will occur unless a specific "response" or "melt" temperature has been exceeded, and the magnitude of the response will be proportional to the length of time the setpoint temperature is exceeded. Indicator "C" records temperature exposure above its "response" temperature with a diffusion of colored fluid along a paper wick, and the "A" and "D" indicating devices record exposures above their "melt" point with the displacement of a thawing capillary of frozen liquid. These indicators vary in sensitivity to temperature increases above the "response" temperature.

The "B" device may be considered a full-history time-temperature indicator, as its response is independent of a temperature threshold. Indicator "B" responds with a gradual color change brought about with a pH-indicator, in solution with an enzyme-substrate mixture, which changes color because of pH changes caused by enzymatic hydrolysis of the lipid substrate. The enzyme reaction is irreversible and an increase in temperature accelerates the reaction thus speeding the color change. The indicator response is given by four discrete color increments, namely, 0, 1, 2, and 3.

Temperature treatments

Two constant temperature storage conditions were chosen for this investigation, −18°C and +5°C. Also a variable temperature cycle, consisting of −18°C for 72 hr followed by +20°C for 1 hr and +5°C for 8 hr was investigated. The duration of the investigation was limited to 14 days. These particular treatments were chosen as a model of the conditions encountered in frozen food transport and handling.

The indicators were attached to an aluminum-sheet plate (33.02 cm × 15.24 cm × 0.48 cm) with a type-T thermocouple placed in a milled slot at the geometric center. Plates which were to be stored in cold storage (−18°C exposure) were placed in a well equilibrated insulated container internally fitted with a plywood support structure. The plates were held vertically within the insulated container and remained enclosed inside the container at all times except for a short period of time during inspection. A cutaway sketch of the experimental setup is shown in Fig. 1.

The temperature exposures of +5°C and +20°C were accomplished in an AMINCO Aire, air conditioning unit. Aluminum was chosen as the indicator mounting surface because the thermal properties of this material would allow rapid equilibration to the exposure temperatures.

Indicator Activation and Evaluation

Indicators "A" and "D" were preconditioned according to manufacturers' specifications by storing them at −18°C for 12 hr before activation. Preconditioning was necessary for these indicators as their principle of operation is based on the melting of a capillary of fluid. Indicator "C" required no preconditioning, but was allowed to equilibrate to the treatment temperatures before activation to help eliminate the variations in the starting points. For the "B" indicators the manufacturer's instructions specifically called for the indicators to be activated above 0°C.

The activated indicators were placed in the various treatment locations and inspected periodically. The indicators which were subjected to the warmest temperature treatments required nearly continuous monitoring, while the coldest treatment required inspection on a 12-hr interval. It was the intent of the inspections to record indicator scale readings and observe any performance limitations. To minimize the indicator exposure during inspection, the devices were examined either within the cold storage freezer or through a plexiglass viewing port in the air conditioning unit.

RESULTS & DISCUSSION

Time-temperature response

Measurement of time-temperature response. All figures are presented as plots of "Percent of Full Scale (%) vs. Exposure Time (hours)." The "Percent of Full Scale" values were mathematically calculated from the averages of the valid indicators' readings at the various temperature treatments. The various indicator scales were normalized such that an expiration reading or endpoint value corresponded to a 100% full scale reading. Thus, for example, for indicator "B", the discrete color increments 0, 1, 2, 3 would correspond to percent of full scale readings of 0%, 33%, 67%, and 100%, respectively. Intermediate percentage readings between these discrete intervals arose as a result of the arithmetic mean of several indicators at any given inspection time. These calculations were necessary for the direct comparison between the indicators from different manufacturers.
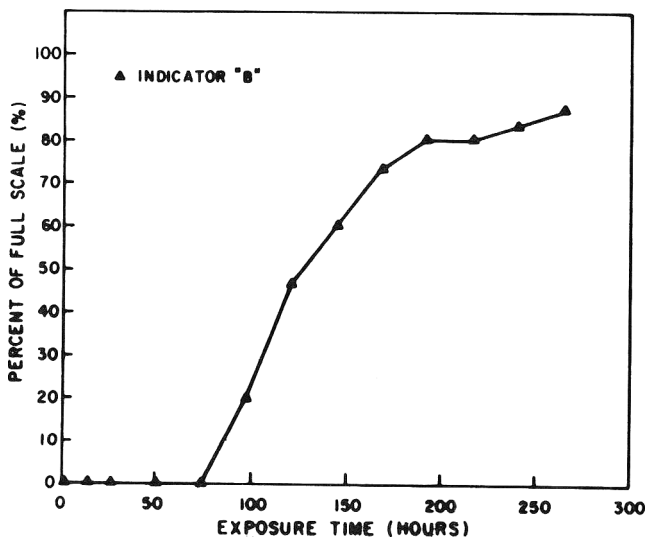
Response to constant temperature treatment. The response of indicator "B" at $-18°C$ constant storage is shown in Fig. 2. The response of indicators "A", "C", and "D" were not included as the $-18°C$ storage temperature was lower than the indicators' threshold temperatures. A small number of indicators of types, "A", "C", and "D", did respond initially, but these indicators yielded no sustained response, and the subsequent flat response was merely an initial offset. It is most probable that this initial offset came about because of warming while the indicators were being activated, and not because of the storage condition or indicator variability.

The response of all indicators at $+5°C$ are shown in Fig. 3. To accommodate a common starting point, all indicators were precooled at $-18°C$ and then suddenly exposed to the $+5°C$ environment. This was necessary to maintain the frozen status of the fluid within the capillaries in indicators "A" and "D" so as not to bias their response. Indicator "D" responded in a strict linear manner, almost immediately after exposure to $+5°C$. The response of indicators "A" and "C" leveled to an approximately linear rate after a relatively short warming period. Indicator "B" responded with a sigmoidial curve, as expected, with a much steeper slope than for the lower temperature exposure.

Response to variable temperature treatment. The response of indicator "C" to the variable treatment is shown in Fig. 4. All indicators except the "C" device expired during the first high temperature exposure and, for this reason, are not included in this figure. Indicator "C" did not reach full scale response until well after the second variable cycle. During the $+20°C$ step, the "C" indicators responded very rapidly and then, unexpectedly continued to respond even when moved back to the $-18°C$ storage condition. Although the $-18°C$ temperature was less than the "response" temperature claimed by the manufacturer, it is suspected this continued response (creeping effect) is a result of a combination of temperatures close to the indicators' response temperature and a continued, but slowed, diffusion of the fluid already in solution within the paper wick.
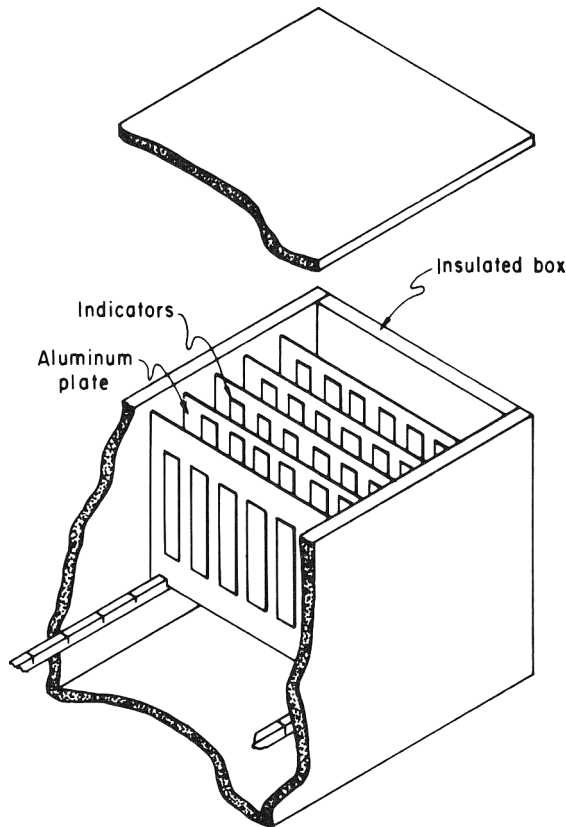

Fig. 1—Low temperature setup to evaluate time-temperature indicators.

Fig. 2—Time response of indicator "B" at $-18°C$ constant storage.
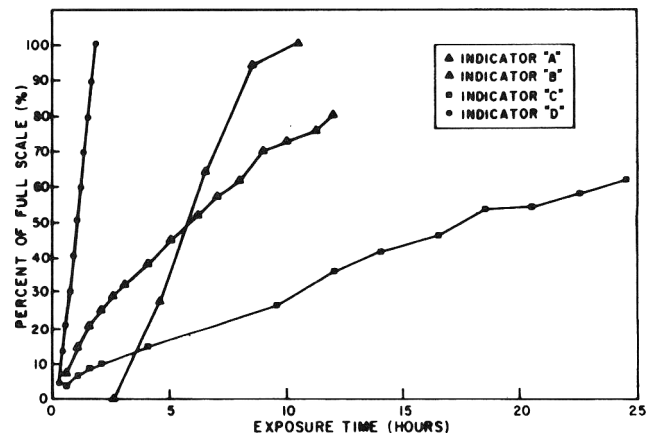

Fig. 3—Time response of all indicators at $+5°C$ constant storage.

Indicator expiration

A tabular summary of the average time to expiration for each indicator type and the coefficient of variability at expiration during the constant temperature exposures is presented in Tables 1 and 2, respectively. The average time to expiration was derived by considering the length of time it took individual indicators to achieve a full scale reading. For this reason, only indicators which had reached full scale were included in this calculation.

Performance assessments

A comparison of the indicator performance characteristics is given in Table 3. The most serious limitation with the use of time-temperature indicators is the great deal of indicator manipulation and meticulous hand work necessary to accomplish indicator activation and attachment.

The scale on which response is recorded for some of the indicators is somewhat tedious. The scale on indicator "B" is especially troublesome for quantitative interpretations, as the color change response is restricted to four discrete scale readings as indicated by a comparator color reference attached to the indicator body. The color scale is further complicated by its dependence on the prevailing lighting conditions in the inspection area, and by the perception of the viewer.

Indicator "A" is mounted to a heavy paper card which fits into an adhesive backed clear plastic pouch. The indicator and card must be removed to be activated and then returned to the pouch for storage placement and mounting. Indicator "B" and "C" are adhesive backed and can be directly mounted for storage. The adhesive of indicator "C" would not adhere to the aluminum plates and it was necessary to attach these indicators with the aid of small pieces of common duct-tape. Indicator "D" is relatively large compared to the other devices and does not have an independent means of attachment.

A large number of the "A" indicators tested registered invalid readings as a result of capillary fluid separation. This was apparently due to the presence of residual gas in the fluid which generated bubbles within the capillary causing voids and separation. One of the "D" indicators tested responded with a mid-scale reading upon activation, a result of an improperly filled capillary. The low level of frozen fluid within the capillary did not allow the indicating probe to be in line with the zero scale reading.

SUMMARY

SINCE $-18°C$ is the recommended maximum temperature exposure during frozen food transport, the results of this study would imply that any of the time-temperature indicators investigated could be used as temperature history indicators during frozen food transport. Indicators "A", "C" and "D" respond only to temperatures above their respective threshold temperatures, while indicator "B" gives a cummulative response over its entire time-temperature history. The use of indicators "A" and "D" should be limited to monitoring shorter duration frozen shipments because of their rapid expiration at $+5°C$, while indicators "B" and "C" would be suitable for somewhat longer shipment durations. At this point in the development of time-temperature indicator technology all indicator types seem suitable for monitoring frozen food transport, but because of the relatively high degree of indicator response variability, sound statistical sampling and inspection procedures must accompany any implementation of time-temperature indicators.

REFERENCES

Arnold, G. and Cook, D.J. 1977. An evaluation of the performance claimed for chemical time-temperature integrating devices. J. Food Technol. 12(4): 333.
Byrne, C.H. 1976. Temperature indicators — the state of the art. Food Technol. 30(6): 66.

Table 1—Average time to expiration (hr) for constant temperature treatments

Indicator	$-18°C$	$+5°C$
Indicator "A"	NR[a]	NR
Indicator "B"	163.50 hr	8.77 hr
Indicator "C"	NR	59.57 hr
Indicator "D"	NR	0.84 hr

[a] NR indicates no response recorded.

Table 2—Coefficient of variability for indicators at expiration during constant treatment

Indicator	$-18°C$	$+5°C$
Indicator "A"	—	—
Indicator "B"	32.34%	7.37%
Indicator "C"	—	5.48%
Indicator "D"	—	1.19%
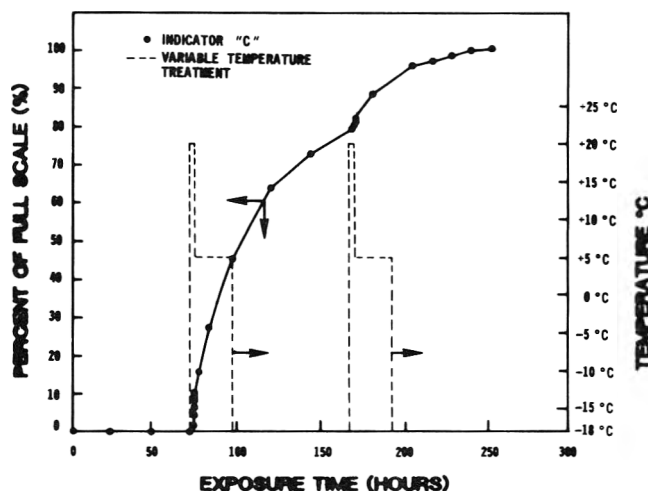
Fig. 4—Time-temperature response of indicator "C" to variable temperature treatment.

Table 3—Indicator performance characteristics

	"A"	"B"	"C"	"D"
Classification	Partial history	Full history	Partial history	Partial history
Response mechanism	Fluid capillary	Enzyme reaction	Liquid diffusion	Fluid capillary
Response scale	Linear 0 - 6 hr	Color references 0, 1, 2, 3	Logarithmic 0 - 10	Linear 0 - 1000 (degree-min)
Means of activation	Hand	Hand/mechanical	Hand	Hand
Preconditioning	Yes	No	Yes/No	Yes

Effects of Gamma-Radiation on Cooking Quality and Sensory Attributes of Four Legumes

V. S. RAO and U. K. VAKIL

─────────── ABSTRACT ───────────

Effects of gamma-radiation (1-10 kGy) on selected functional properties were studied in four legumes: green gram, lentil, horse-bean and Bengal gram. Water absorption capacity of irradiated legume samples increased, although pasting temperature was not appreciably changed. Maximum gelatinization viscosity decreased progressively in all samples with increasing radiation dose. Irradiation at 2.5-10.0 kGy of all legume samples caused a significant reduction in cooking time compared to controls. The force required to compress irradiated cooked samples was reduced. Irradiated samples (5 kGy) were rated as softer than control and 2.5 kGy sample by taste panel members. Sensory evaluation of cooked unirradiated and irradiated (5 kGy) samples revealed no significant differences in acceptability.

INTRODUCTION

LEGUMES are rich sources of easily available, cheap proteins and complement cereal proteins in terms of several essential amino acids, when blended in the right proportions (Liener, 1978). Their optimum utilization may help in combating the prevailing protein-calorie malnutrition conditions in developing countries. However, their acceptability as staple foods is limited because they contain several heat stable as well as heatlabile antinutritional factors (Liener, 1979). Some of these, on ingestion of legumes, may cause gastro-intestinal discomfort. The prolonged time generally required for cooking also makes their utilization uneconomical. Radiation treatment at moderate doses has been recommended for disinfestation of legumes (Food Irradiation Information, 1980). The treatment is shown to be effective for reduction of flatulence-causing oligosaccharides (Rao and Vakil, 1983) and also of trypsin and chymotrypsin inhibitors (Iyer et al., 1980b). Further, the nutritive quality of irradiated beans is improved (Reddy et al., 1979). Short term feeding studies in rats fed irradiated bean diets do not reveal any mutagenic activity by *in-vivo* nor *in vitro* tests (Food Irradiation Information, 1980). However, some subtle physicochemical changes in macronutrients are observed in irradiated foods. Several low molecular weight radiolytic breakdown products of irradiated legume starch (Nene et al., 1975b) and proteins (Nene et al., 1975a) have been characterized. These changes may affect the functionality of the irradiated legumes. In the present investigation, the effects of gamma-radiation, at moderate doses (1-10 kGy) on some of the rheological properties, water absorption capacity, cooking quality and sensory acceptability were studied in four varieties of commonly used legumes.

MATERIALS & METHODS

SAMPLES OF LEGUMES, green gram (*Phaseolua aureus*), horsebean (*Vicia faba*), lentil (*Lens esculenta*) and Bengal gram (*Cicer*

arietinum), were purchased in a single lot from a local market and stored at 4°C in air-tight bins.

Irradiation

The seeds (100g) were packed in individual polythene bags and irradiated (1-10 kGy dose levels) in air at 25°C, in a ^{60}Co Gammacell 220 (BARC Model), having an influx of 0.025 kGy/min and overdose ratio of about 10%. Absorption of ionizing radiation was checked with ferrous sulfate and ceric sulfate dosimetry (Weiss, 1952).

Water absorption

Samples of whole legume seeds were soaked in distilled water (1:5 w/v legume to water ratio) for 1, 3, 6, 12, and 16 hr at room temperature (25 ± 2°C). Excess water was drained and the samples were air dried (30 min) and weighed. The increase in weight was taken as the amount of water absorbed. Percentage increase in original weight of seeds was calculated.

Gelatinization viscosity

Seeds were ground in an experimental roller grinding mill and passed through a 60 mesh sieve. The gelatinization viscosity was measured in a Brabender amylograph. The flour (50g) was mixed with distilled water (480 mL) to form a lump-free slurry. This was heated up to 95°C in a revolving container (75 rpm) with a constant temperature rise (1.5°C/min); changes were recorded on a chart. No amylogram peak was obtained with legume flour. Therefore, the amylogram reading at 95°C was taken as the maximum gelatinization viscosity (Morad et al., 1980). The temperature at which the viscosity of the slurry began to rise (pasting temperature) was also noted.

Determination of softness on cooking

Legume seeds (10g lots) were cooked for 10-60 min in boiling water (1:10 w/v) containing 2% common salt and 0.2% turmeric powder. The extent of softening during cooking was measured with an 'Instron' Universal texturometer (table model), consisting of a moving cross head and adjusted to a maximum pressure of 100 kg. The cooked sample was kept in a specially devised cylindrical aluminium cup and compressed at a cross-head speed of 20 mm/min; chart speed was 200 mm/min. The peaks, indicating the force required (kg) to compress the samples, were recorded. In another experiment, time required for complete cooking (as determined by subjective test) of control legumes were first determined. The irradiated samples were then cooked for the same time and the force required to compress both the control and irradiated legumes was determined. Reduction in cooking time after irradiation was calculated from these values.

Sensory evaluation

Irradiated (1−5 kGy) horsebeans and Bengal gram were cooked in water for 60 min and lentils and green gram for 10 min, the optimum time required for cooking by their respective unirradiated controls. Spices were added as described above. Excess water was drained and the cooked samples were kept in a hot cabinet (75-80°C) until served. Overall acceptability was evaluated by a trained taste panel of 12 members using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). Each sample was tested three times on different days. An intensity scale (1 = none to 5 = extreme) was used for scoring off-characterisitcs such as discoloration, off-odor and irradiation flavor. Legumes, irradiated at 10 kGy, had a distinct discoloration and irradiation flavor, hence, these samples were not served to the taste panel. Means and standard error were calculated for each characteristic as well as for hedonic scale scores.

Statistical comparisons were made using the Student's t-test (Snedecor and Cochran, 1976). Differences in mean values yielding $P \leqslant 0.05$ were considered significant.

RESULTS

Water absorption

Hydration capacity of all the seeds was maximum at 1 hr and then leveled off (data not shown). A typical histogram (Fig. 1) depicts the effect of radiation treatment (10 kGy) on water absorption capacity of various legumes soaked for 1 and 16 hr in water. After 1 hr of soaking, all the irradiated samples showed higher extent of hydration than their respective controls. This difference in the water uptake capacity between irradiated and control samples was marginal (5%) for green gram, whereas, it was much larger (15, 20 and 27%, respectively) for Bengal gram, lentil and horsebean. These percent differences in the extent of hydration between the irradiated and control samples were maintained after 16 hr of soaking. At lower doses, radiation treatment did not significantly affect the water absorption capacity of the legumes.

Evaluation of amylgram

The pasting temperature of unirradiated legumes was in the range 70-75°C for green gram, lentil, Bengal gram and horsebean (Table 1). This was decreased appreciably in Bengal gram (by 10°C) and green gram (by 7°C) samples, irradiated at 10 kGy dose levels but not in lentil (4°C) and horsebean (2°C). Since no distinct peak was obtained with legume flours, as is normally found with wheat flour or isolated cereal starch, the maximum gelatinization viscosity obtained when the temperature reached 95°C, was recorded (Fig. 2). Maximum gelatinization viscosity (A.U.) at 95°C of green gram (235), lentil (475), Bengal gram (315) and horsebean (200) decreased progressively in all the samples with increasing dose of radiation.

Cooking quality of irradiated legumes

The softness of the cooked legume samples was measured with an 'Instron' texturometer. A typical set of results obtained with control and irradiated horsebean are depicted in Fig. 3A. Control sample, considered cooked on the basis of subjective evaluation by a taste panel, took 60 min for complete cooking and required a kg force of 70 to compress. However, the force (kg) required for compres-

sion of completely cooked control samples of other legumes varied: 80 for lentil, 65 for green gram and 40 for Bengral gram (Fig. 4). Tracings of original Instron peaks (Fig. 3B) revealed that irradiated horsebean samples, cooked for the time required to obtain the desired softness of the control (60 min) were much softer than the control. At 5 and 10 kGy irradiation, forces of only 42 and 39 kg, respectively, were required to compress the horsebeans. The cooking time for the irradiated samples, calculated using the Instron values, was found to be reduced in all legume samples, when compared with their control. Thus, it was calculated that the horsebean samples irradiated at 2.5, 5.0 and 10 kGy, took 52, 50 and 35min, respectively, for cooking as compared to 60 min for control. The reduction in cooking time of irradiated legumes compared to their respective control was plotted against the radiation dose (Fig. 5). Maximum reduction was observed in green gram irradiated at 5 (35%) and 10.0 (50%) kGy dose levels. At 5.0 kGy lentil showed appreciable reduction (28%), whereas, horsebean and Bengal gram showed only 13 – 16% reduction. At 10 kGy, however, 40 – 50% reduction was recorded in cooking time of all the legumes tested.

Table 1 — Measurement of the pasting temperature of irradiated legumes, evaluated from amylograms

| Legume | Irradiation dose (kGy) | | | |
	0	2.5	5.0	10.0
	Pasting temp [a] (°C)			
Green gram	75	74	72	68
Lentil	70	69	68	66
Horsebean	72	72	71	70
Bengal gram	72	70	68	62

[a] Initial pasting temperatures (°C) of various legume flours were measured in a Brabender amylograph as described in the text. Results are averages of four independent experiments.

Fig. 1—Effect of radiation treatment on hydration capacity of four legum samples. ■, control; ▨, irradiated at 10 kGy.
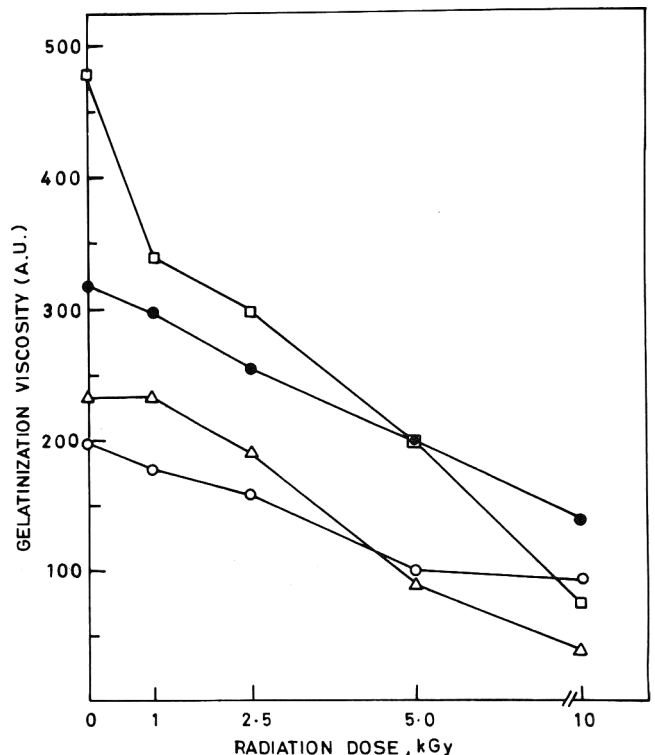
Fig. 2—Evaluation of amylograms of irradiated legumes: △ — △, green gram; ▢ — ▢, lentil; ○ — ○, horsebean; ● — ●, Bengal gram.
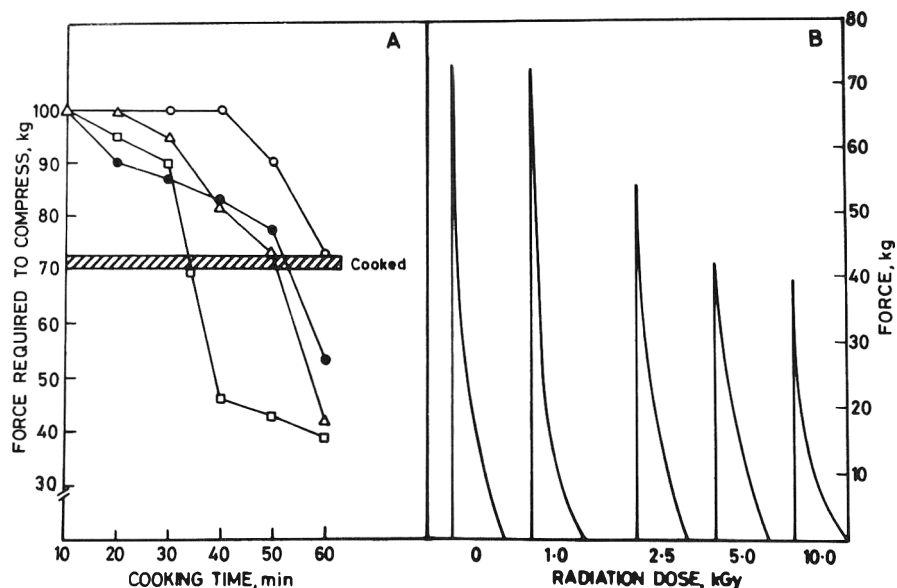
Fig. 3—Compression of control and irradiated cooked horsebean in an Instron texturometer. Force required to compress the legumes cooked for different time intervals: (A) Instron peaks of control and irradiated horsebean samples cooked for 60 min (B). ○ — ○, control; ● — ●, 2.5 kGy; △ — △, 5.0 kGy; □ — □, 10 kGy.

Sensory evaluation

Average scores of acceptability of cooked unirradiated and irradiated legume samples on a 9-point hedonic scale are given in Table 2. Cooked samples at 2.5 kGy dose, except in green gram, showed significantly lower rating (P < 0.05) on hedonic scale as compared to the control. However, at 5.0 kGy dose, as compared to the control, there was no significant difference in acceptability in all cooked samples. Further, remarks about the softness of the cooked samples were evaluated. About 72 – 100% of the panel members were of the opinion that the texture of the samples irradiated at 5 kGy was much softer and smoother on cooking than that of the control or of 2.5 kGy samples. Evaluation of undesirable off-characteristics (Table 3) showed that intensity of discoloration, off-ocor and irradiation flavor increased as a function of radiation dose. However, even at 5 kGy, only marginal differences in scores of irradiation flavor (1.75 – 2.0) or discoloration (1.9 – 2.2) was noticed.

DISCUSSION

WATER ABSORPTION CAPACITY of whole legumes (Fig. 1) is generally lower than that of wheat (Rao et al., 1978) or corn (Vose, 1977) due to lower permeability of hard seed coats and to a close association of starch polymers in the native granules of legumes (Lorenz, 1979). However, the hydration rate of the irradiated legumes was increased. Nene et al. (1975b) have carried out the experiments with red gram, under the identical conditions and using the same radiation dose as used in this study. They have shown that solubility of irradiated (10 kGy) legume flour or of isolated starch was increased with a concomitant increase in reducing sugars. When these samples were subjected to in vitro α-amylase action, relatively more maltose was liberated from irradiated samples. Similarly, an increase in free
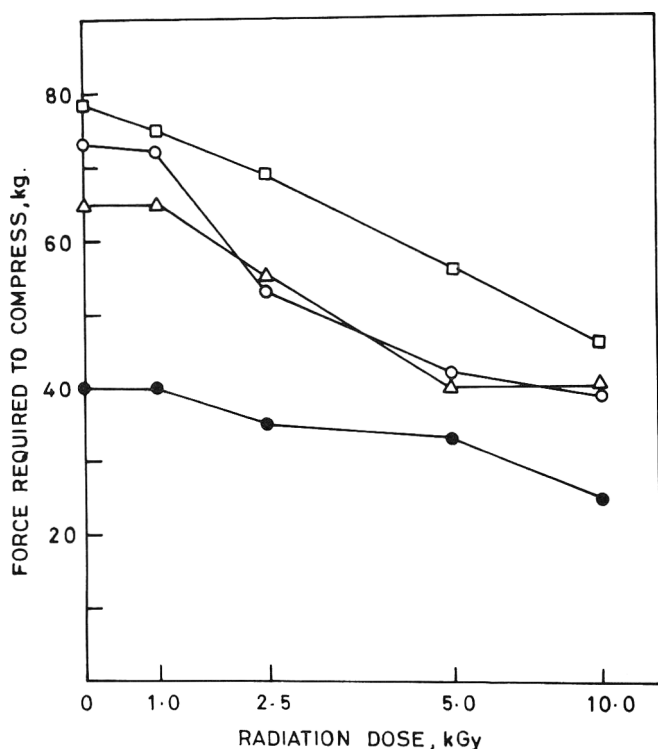
Fig. 4—Measurement of softening in irradiated cooked legumes. Irradiated (1 - 10 kGy) legumes were cooked for the optimum cooking time required by their respective controls: △ — △, green gram; □ — □, lentil; ○ — ○, horsebean; ● — ●, Bengal gram.
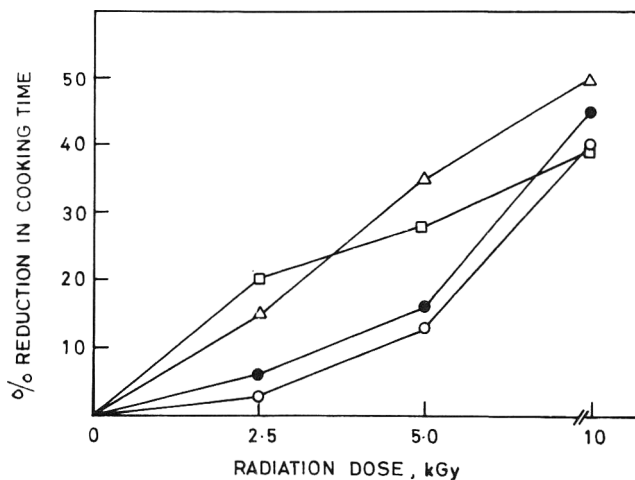
Fig. 5—Effect of γ-radiation on percent reduction in cooking time of the legumes: △ — △, green gram; □ — □, lentil; ○ — ○, horsebean; ● — ●, Bengal gram.

amino acid levels in soluble proteins and in vitro proteolysis were observed (Nene et al., 1975a) in irradiated (10 kGy) red gram compared to their unirradiated control. The radiosensitivity of isolated starch was more than that of flour, where starch is present in more complex form in association with others. Thus, increase in water uptake may be attributed to breakdown of starch and protein molecules to low molecular weight entities and to their increased solubility in irradiated samples. It is known that physically damaged smaller particle sized starch helps in increasing the available surface area of hydration (Meredith, 1966). Further, the differences in the rate of hydration of the legumes studied can be explained by the heterogenity of their cell wall composition, molecular size and natural binding of the individual sugar components within the cells of each sample (Sathe and Salunkhe, 1981). These factors are shown to govern the apparent hydration of legumes during soaking (Shun Ku et al., 1976). Further, the decrease in initial pasting temperature (Table 1) and gelatinization viscosity (Fig. 2) were observed in irradiated legume samples. Since these properties are mainly governed by the length of polysaccharide chains (Reddy et al., 1984), it could be inferred that starch molecules in these legumes were shortened or depolymerized due to radiation treatment. Appreciable reduction in cooking time of the irradiated legumes was observed (Fig. 5) although different times for complete cooking in terms of softness (Fig. 4) were noted. Irradiation at 5 kGy of soaked and dehydrated beans causes about 50% reduction in cooking time (Iyer et al., 1980a). Williams et al. (1983) observed a direct relationship between cookability, swelling index, and hydration capacity of the soaked seeds. Excessive cooking of the legumes results in protein losses and in lower availability of lysine (Almas and Bender, 1980). Cooking time can be reduced by addition of alkaline chemicals. However, this adversely affects the overall acceptability of the cooked samples (Narasimha and Desikachar, 1978) and also produces unnatural cross-linked amino acids (e.g. lysinoalanine), having nephrotoxic effects on rats fed such diets (Pfaender, 1983). On the other hand, radiation treatment improves the nutritive and metabolizable values of lentil as observed on the basis of chick bioassay test

(Daghir et al., 1983). Sensory evaluation on either a hedonic or an intensity scale has shown no significant difference in acceptability of control or irradiated (at 5 kGy) legumes. Irradiated samples (though had slight discoloration at 5 kGy dose) were more acceptable because they were very soft. At 2.5 kGy, the samples, although they did not have off-characteristics, scored less than the control or 5 kGy samples because these were not so soft in texture. The overall scores (hedonic scale, Table 2) were in the lower range. This may be attributed to blandness of the samples, cooked without addition of the conventional spices. Urbanyi (1982) has observed that food, served in a form not normally consumed, always scores lower.

REFERENCES

Almas, K. and Bender, A.E. 1980. Effect of heat treatment of legumes on available lysine. J. Sci. Food Agric. 31: 448.
Daghir, N.J., Sell, L.J., and Mateos, G.G. 1983. Effect of gamma irradiation on nutritional value of lentils (Lens culinaris) for chicks. Nutr. Rep. Intern. 27: 1087.
International Project in the field of food irradiation news 1980. In "Food Irradiation Information," Vol. 10, (Ed.) Potter, W.T., Elias, P.S., and Gottschalk, H.M., p. 46. Karlsruhe, Germany.
Iyer, V., Salunkhe, D.K., Sathe, S.K., and Rockland, L.B. 1980a. Quick-cooking beans (Phaseolus vulgaris L.): 1. Investigations on quality. Qual. Plant. Plant Foods Human Nutr. 30: 27.
Iyer, V., Salunkhe, D.K., Sathe, S.K., and Rockland, L.B. 1980b. Quick-cooking beans (Phaseolus vulgaris L.): 2. Phytates, oligosaccharides and antienzymes. Qual. Plant. Plant Foods Human Nutr. 30: 45.
Liener, I.E. 1978. Toxins in legumes: Their possible nutritional significance in the diet of man. In "Quality attributes of edible plant tissues," p. 348. Academic Press, New York.
Liener, I.E. 1979. The nutritional significance of plant protease inhibitors. Proc. Nutr. Soc. 38: 109.
Lorenz, K. 1979. The starch of fababean (Vicia faba), comparison with wheat and corn starch. Die Starke 31: 181.
Meredith, P. 1966. Dependence of water absorption of wheat on protein content and degree of starch granule damage. New Zealand J. Sci. 9: 324.
Morad, M.M., Leung, H.K., Hsu, D.L. and Finney, P.L. 1980. Effect of germination on physico-chemical and bread making properties of yellow pea, lentil and faba bean flours and starches. Cereal Chem. 57: 390.
Narasimha, H.V. and Desikachar, H.S.R. 1978. Simple procedures for reducing the cooking time of split red gram (Cajanus cajan). J. Food Sci. Tech. 15: 149.
Nene, S.P., Vakil, U.K., and Sreenivasan, A. 1975a. Effect of gamma-radiation on red gram (Cajanus cajan) proteins. J. Food Sci. 40: 815.
Nene, S.P., Vakil, U.K., and Sreenivasan, A. 1975b. Effect of gamma-radiation on physico-chemical characteristics of red gram (Cajanus cajan) starch. J. Food Sci. 40: 943.
Pfaender, P. 1983. Lysinoalanine — A toxic compound in processed proteinaceous foods. Wold Rev. Nutr. Dietet. 41: 97.
Rao, V.S., Vakil, U.K., and Sreenivasan, A. 1978. Studies on rheological and baking properties of irradiated Indian wheat. Acta Aliment. 7: 91.
Rao, V.S. and Vakil, U.K. 1983. Effects of gamma-radiation on flatulence-causing oligosaccharides in green gram (Phaseolus areus). J. Food Sci. 48: 1791.
Reddy, N.R., Pierson, M.D., Sathe, S.K., and Salunkhe, D.K. 1984. Chemical, nutritional and physiological aspects of dry bean carbohydrates — A review. Food Chem. 13: 25.
Reddy, S.J., Pubols, M.H., and McGinnis, J. 1979. Effect of gamma irradiation on nutritional value of dry field beans (Phaseolus vulgaris) for chicks. J. Nutr. 109: 1037.
Sathe, S.K. and Salunkhe, D.K. 1981. Isolation, partial characterization and modification of the great northern bean (Phaseolus vulgaris L) starch. J. Food Sci. 46: 617.
Shun Ku, L.S., Wei, L.S., Steinberg, M.P., Nelson, A.I., and Hymowitz, T. 1976. Extraction of oligosaccharides during cooking of whole soyabeans. J. Food sci. 41: 361.

Table 2 — Sensory evaluation (acceptability) of irradiated cooked legumes

Legume	Irradiation dose (kGy)		
	0	2.5	5.0
		Mean score on scale[a]	
Green gram	6.43 ± 0.06	6.34 ± 0.01	6.45 ± 0.02
Lentil	6.32 ± 0.11	5.85 ± 0.12*	6.00 ± 0.14
Horsebean	6.22 ± 0.10	5.54 ± 0.04*	6.02 ± 0.03
Bengal gram	6.63 ± 0.10	6.09 ± 0.13*	6.33 ± 0.14

[a] Control and irradiated cooked samples were evaluated for acceptability by a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). Mean score with standard error (±) is calculated from three taste panel results.
* P < 0.05.

Table 3 — Evaluation of off — characteristics of control and irradiated cooked legumes

Legume	Average score on intensity scale[a]								
	Irradiation dose (kGy)								
	0	2.5	5.0	0.0	2.5	5.0	0	2.5	5.0
	Discoloration			Off-odor			Irradiation flavor		
Green gram	1.27	1.67	2.15	1.20	1.40	1.97	1.30	1.52	2.00
Lentil	1.60	1.80	1.90	1.30	1.60	1.75	1.30	1.75	1.95
Horsebean	1.40	1.70	2.00	1.10	1.40	1.70	1.20	1.50	1.75
Bengal gram	1.50	1.90	2.00	1.20	1.60	1.70	1.10	1.35	1.94

[a] Intensity scale — 1, none; 2, slight; 3, moderate; 4, strong; 5, extreme. Results are averages of three panel tests.

Evaluation of Substrate Potentiality and Inhibitory Effects to Identify High Risk Spices for Aflatoxin Contamination

M. S. MADHYASTHA and R. V. BHAT

ABSTRACT

Growth and aflatoxin production by *A. parasiticus* (NRRL 2999) on autoclaved whole, ground and also surface sterilized black pepper, cardamom, red pepper, dry ginger and turmeric were studied. Cardamom did not support detectable fungal growth or aflatoxin production. Black pepper and turmeric appeared to be poor substrates as they supported comparatively less fungal growth and aflatoxin production. Red pepper and ginger were found to be better substrates for fungal growth as well as for aflatoxin production. Ether and chloroform extracts of cardamom and turmeric inhibited aflatoxin production almost completely. The inhibitory activity of cardamom oil and curcumin indicated that they might be the active principles.

INTRODUCTION

SINCE ANTIQUITY, spices have been used for flavoring foods and beverages and for medication. They are also highly valued for their use as preservatives and antioxidants. There is a considerable volume of international trade in spices amounting to about 365 million dollars annually and hence they play an important role in the national economy of several of the producing, exporting and importing countries (Pruthi, 1980). Tropical climatic conditions under which these spices are grown offer a favorable environment for the fungal contamination. The natural occurrence in various spices of *Aspergillus flavus* and its metabolite aflatoxin, a well known hepatotoxic, mutagenic, carcinogenic and teratogenic agent, have been summarised by Udagawa (1982). Aflatoxin has been produced in some spices under artificial conditions (Flanningan and Hui, 1976; Seenappa and Kempton, 1980; Llewellyn et al., 1981a, b). Although the toxin production under laboratory conditions is not directly comparable to that occurring under natural conditions, these observations may be relevant to some degree to the problem of aflatoxin production under natural conditions.

The purpose of the present study was to evaluate the substrate suitability of a number of major spices for the growth and aflatoxin production of *Aspergillus parasiticus* Speare (NRRL-2999) under different experimental conditions. Further, an attempt was made to study the effect of ether and chloroform extracts of cardamom and turmeric and also cardamom oil and curcumin, a coloring principle of turmeric on fungal growth and aflatoxin production.

MATERIALS & METHODS

Organism

The organism used in this study was *A. parasiticus* Speare (NRRL-2999) and the cultures of the organism were maintained on potato dextrose agar slants at 28°C ± 2°C.

Inoculum

The inoculum was produced by growing the organism on potato

dextrose agar slants for 4 days at 28 ± 2°C. The conidia were harvested by adding 10 ml sterile water and aseptically dislodging the conidia with a sterile inoculating loop. The conidial suspension thus obtained had conidial concentration of 10^6 conidia/mL.

Substrate

Whole and ground black pepper (*Piper nigrum* L.), cardamom (*Elettaria cardamomum* L.), red pepper (*Capsicum annuum* L.), dry ginger (*Zingiber officinale* Roscoe) and turmeric (*Curcuma longa* L.) samples (50g each) were taken in 500 mL Erlenmeyer flasks in replicates. A set of whole and ground samples were autoclaved at 121°C under 15 psi for 15 min while another set of whole samples was surface sterilized with 0.1% $HgCl_2$. All the samples were inoculated with conidial suspension of *A. parasiticus* and incubated at 28 ± 2°C for 7 days. Uninoculated samples served as controls. The semi-synthetic medium used for testing the effect of ether and chloroform extracts of cardamom and turmeric and also cardamom oil and curcumin consisted of 2% yeast extract and 15% sucrose (YES) broth which is known to support aflatoxin production (Davis et al., 1966). The broth was dispensed in 50 mL quantities into 250 mL Erlenmeyer flasks and sterilized by autoclaving at 121°C for 15 min. Ether and chloroform extracts of cardamom were obtained separately by extracting 5g, 10g and 25g ground cardamom with 25 mL, 50 mL and 125 mL of each solvent, respectively. Similar extracts were obtained from turmeric also. Extracts were filtered through Whatman No. 1 filter paper and evaporated to dryness on a boiling water bath. The residue of each was dissolved in 2 mL each dimethyl sulfoxide (DMSO) and transferred into flasks under aseptic conditions. Cardamom oil emulsified with 0.5 mL Tween '80' was added to liquid medium to give concentrations of 50, 100, 200, 300 and 500 µL/50 mL. Curcumin, dissolved in DMSO, was added to medium to obtain the concentrations of 10, 100, 500, 1,000 and 10,000 µg/mL before sterilization. The inoculum was added to each flask at the rate of 0.5 mL/flask and incubated at 28 ± 2°C for 7 days. Controls were maintained for the different treatments. Cardamom oil and curcumin were obtained from Central Food Technological Research Institute, Regional Station, Hyderabad, and Central Drug Research Institute, Lucknow, India, respectively.

Fungal growth and aflatoxin production

Fungal growth on whole and ground spice substrates was estimated in terms of chitin as per the procedure described by Ride and Drysdale (1972). Aflatoxins were extracted by the modified method of Suzuki et al (1973). In the modified method a neutral alumina layer was included in the column for the clean up. Instead of two dimensional thin layer chromatography, the plate was developed in chloroform + acetone (95:5 v/v) and anhydrous diethyl ether, successively. Quantitation was done by Photovolt densitometer (Pons et al., 1966).

Mycelial mat from the liquid medium in each flask was separated by filtering through Whatman No. 1 filter paper. Mats were washed twice with distilled water and dried at 80°C for 16 hr to a constant weight. the filtrates were extracted with 25 mL chloroform three times, the extracts were combined and evaporated to near dryness. The residue was dissolved in 1 mL benzene + acetonitrile (98:2 v/v) and analyzed qualitatively by TLC and quantitatively by Photovolt densitometer (Pons et al., 1966).

RESULTS

AMONG the major spices tested, cardamom did not support detectable fungal growth and aflatoxin production. Black pepper and turmeric appeared to be poor substrates for fungal growth as well as aflatoxin production. Ground

Table 1—Growth and aflatoxin production of Aspergillus parasiticus on major spices[a]

Spice	Autoclaved (whole)			Surface Sterilized (whole)			Autoclaved (Powder)		
	Dry wt of Mycelia (mg/g)	AFB_1 (μg/g)	AFG_1 (μg/g)	Dry wt of Mycelia (mg/g)	AFB_1 (μg/g)	AFG_1 (μg/g)	Dry wt of Mycelia (mg/g)	AFB_1 (μg/g)	AFG_1 (μg/g)
Black pepper	10.4 ± 0.6[b]	0.05 ± 0.0	NE	18.4 ± 0.8	0.06 ± 0.0	NE	8.3 ± 0.3	ND	ND
Dry ginger	28.2 ± 3.9	0.63 ± 0.0	0.27 ± 0.0	19.3 ± 3.3	0.4 ± 0.0	0.11 ± 0.0	33.7 ± 1.6	3.7 ± 0.2	0.53 ± 0.0
Red pepper	26.4 ± 1.1	1.8 ± 0.0	0.67 ± 0.0	20.1 ± 2.1	3.6 ± 0.0	1.2 ± 0.0	41.8 ± 2.2	0.05 ± 0.0	0.02 ± 0.0
Turmeric	5.2 ± 0.3	0.03 ± 0.0	Tr.	7.2 ± 0.3	0.04 ± 0.0	Tr.	8.4 ± 0.3	0.04 ± 0.0	0.02 ± 0.0

[a] Cardamon did not support detectable fungal growth and aflatoxin production.
NE: Not Estimated. ND: Not Detected. Tr.: Trace.
[b] Mean value ± SD

Table 2—Effect of ether and chloroform extracts of cardamom and turmeric on growth and aflatoxin production of Aspergillus parasiticus

	Cardamom[a]							Turmeric[a]						
	5g		10g		25g			5g		10g		25g		
	EE	CE	EE	CE	EE	CE	Control	EE	CE	EE	CE	EE	CE	Control
Dry weight of Mycelia (mg/mL)	16.7 ± 1.2[b]	19.8 ± 0.1	11.5 ± 1.5	10.7 ± 0.3	9.3 ± 0.1	ND	47.8 ± 0.6	29.8 ± 3.9	23.1 ± 0.7	26.4 ± 0.9	20.1 ± 2.9	26.4 ± 0.0	19.8 ± 0.6	40.2 ± 0.2
Aflatoxin B₁ (μg/mL)	5.4 ± 0.1	0.24 ± 0.0	Tr.	Tr.	Tr.	ND	48.0 ± 2.8	10.2 ± 0.3	18.5 ± 0.7	6.2 ± 0.5	11.0 ± 0.8	0.7 ± 0.0	4.3 ± 0.4	54.5 ± 2.1
Aflatoxin G₁ (μg/mL)	1.3 ± 0.0	0.22 ± 0.0	Tr.	Tr.	Tr.	ND	45.8 ± 1.4	5.4 ± 0.2	4.1 ± 0.1	3.3 ± 0.2	3.5 ± 0.4	0.32 ± 0.1	1.8 ± 0.4	26.3 ± 0.4
Percentage of inhibition (%)[c]	65 (85)	62 (99.5)	76[d]	75[d]	80[d]	100 (100)	0 (0)	25 (81)	40 (67)	34 (89)	48 (80)	34 (99)	49 (92)	0 (0)

[a] Cardamom and turmeric powder (in g) used for extracting with chloroform and ether.
EE: Ether Extract; CE: Chloroform Extract.
[b] Mean value ± SD
[c] Values in parentheses indicate the inhibition of total aflatoxin production and those outside for fungal growth inhibition.
[d] Inhibition of total aflatoxin production was almost complete.
ND: Not detected. Tr. = Trace.

black pepper inhibited toxin production completely. Ginger and red pepper supported the growth as well as toxin production well. Surface sterilized red pepper was found to support more aflatoxin production when compared to autoclaved substrate. However, ground red pepper allowed comparatively less toxin production, but more fungal growth. Ginger in the ground and toxin production than whole ginger rhizome. Autoclaved whole ginger supported the fungal growth better than surface sterilized whole ginger. There was not much difference in autoclaved and surface sterilized ginger with regard to toxin production. Turmeric in the ground form supported the toxin production slightly better than the autoclaved and surface sterilized rhizomes (Table 1). In the five spices studied, there was no correlation between fungal growth and aflatoxin production.

Ether and chloroform extracts of 25g ground cardamom inhibited fungal growth as well as toxin production almost completely. Ether and chloroform extracts of 25g ground turmeric inhibited fungal growth moderately, but toxin production to a greater extent (Table 2). The inhibitory action of cardamom oil on fungal growth and aflatoxin production was observed to be dose dependent. Although curcumin had stimulatory effect on fungal growth at certain levels, it inhibited aflatoxin production almost completely at higher concentration (Table 3).

DISCUSSION

THE AUTOCLAVED whole black pepper supported aflatoxin production which is similar to that observed by Seenappa and Kempton (1980) after 30 days of incubation. The surface sterilized whole black pepper supported fungal growth as well as aflatoxin production slightly better than the autoclaved one which could be due to the stimulatory effect of essential oil. The total inhibition of toxin production in autoclaved ground black pepper could be due to the exposed pungent principle, piperine (Madhyastha and Bhat, 1984). Ground ginger supported fairly good amount of toxin production. The surface sterilized red pepper appeared to contain some stimulatory substances for aflatoxin production, which might be the volatile oil that is lost dur-

ing autoclaving. But, the ground red pepper which supported comparatively less aflatoxin production appeared to have some inhibitory principle that was exposed in the ground form. The total toxin production on autoclaved whole red pepper was equal to that observed by Flanningan and Hui (1976). As is evident from the study, the inhibitory effect of ether extract of cardamon on fungal growth and aflatoxin production could be due to cardamom oil. Similarly, the inhibitory action of chloroform extract of turmeric on fungal growth and aflatoxin production may be attributed to curcumin and could also be partly due to turmeric oil as the oils of some spices were found to have similar effect (Tiwari et al., 1983).

Although Aspergillus sps. were isolated from cardamom earlier (Pal and Kundu, 1972. Flanningan and Hui, 1976), in the present study it was found to be an unsuitable substrate for the growth and toxin production of A. parasi-

Table 3—Effect of cardamom oil and curcumin on growth and aflatoxin production by Aspergillus parasiticus

Concentration	Dry weight of Mycelia (mg/mL)	Aflatoxin B₁ (μg/mL)	Aflatoxin G₁ (μg/mL)
Cardamom oil (μL/50 mL)			
0	41.5 ± 0.7[a]	42.0 ± 5.7	13.0 ± 4.2
50	37.9 ± 0.3	13.3 ± 0.4	4.0 ± 0.7
100	36.3 ± 0.6	8.7 ± 1.3	3.6 ± 0.9
200	30.4 ± 5.3	6.7 ± 1.6	0.9 ± 0.2
300	24.8 ± 0.7	5.9 ± 0.2	0.8 ± 0.2
500	18.1 ± 2.6	5.3 ± 0.1	0.7 ± 0.1
Curcumin (μg/mL)			
0	30.7 ± 2.7	38.6 ± 9.3	20.6 ± 0.9
10	42.9 ± 1.7	21.5 ± 0.2	11.0 ± 1.4
100	28.2 ± 0.0	7.1 ± 0.1	2.1 ± 0.1
500	28.1 ± 0.1	1.6 ± 0.2	1.8 ± 0.3
1000	17.4 ± 1.0	0.3 ± 0.0	0.04 ± 0.0
10000	30.3 ± 0.1	0.3 ± 0.0	0.03 ± 0.0

[a] Mean value ± SD

ticus. Similar antimycotic and antiaflatoxigenic effects were observed with cinnamon and clove which were later found to be due to their respective chemical components such as cinnamic aldehyde and eugenol (Bullerman et al., 1977). Inhibitory actions of other spices such as thyme, garlic and white mustard on fungal growth and aflatoxin production have been demonstrated (Hitokoto et al., 1977; Mabrouk and El-Shayeb, 1981; Azzouz, 1981). Aflatoxin production by *A. parasiticus* in major spices under laboratory conditions is considerably less when compared to other agricultural commodities such as cereals and oil seeds. Moreover, the amount of consumption of spice by man is low when compared to staple cereals like corn (Madhyastha and Bhat, unpublished). Thus, mycotoxin contamination in spice is unlikely to pose a serious hazard to human health. However, among the spices, ginger and red pepper can be considered as high risk commodities for which routine screening for aflatoxin may be necessary. However, the observations that some of the spices and their extracts are inhibitory to fungal growth and toxin production are important and can be exploited further for containment of toxin contamination of foods.

REFERENCES

Azzouz, M.A. 1981. The inhibitory effects of selected herbs, spices and other plant materials on mycotoxigenic molds. Dissertation Abstracts International, B. 42: 153.

Bullerman, L.B., Leieu, F.Y., and Seier, S.A. 1977. Inhibition of growth and aflatoxin production by cinnamon and clove oils, cinnamic aldehyde and eugenol. J. Food Sci. 42: 1107.

Davis, N.D., Diener, U.L., and Eldridge, D.W. 1966. Production of Aflatoxin B_1 and G_1 by Aspergillus flavus in a semi-synthetic medium. Appl. Microbiol. 14: 378.

Flanningan, B. and Hui, S.C. 1976. The occurrence of aflatoxin producing strains of Aspergillus flavus in the mould floras of ground spices. J. Appl. Bacteriol. 41: 411.

Hitokoto, H., Morozumi, S., Wauke, T., Sakai, S., and Kurata, H. 1977. Mycotoxin production of fungi on commercial foods. In "Mycotoxins in Human and Animal Health," p. 479. (Ed.) Rod-

ricks, J.V., Hesseltine, C.W., and Mehlmann, M.A. Pathotox Publishers, Inc., Park Forest South, IL.

Llewellyn, G.C., Burkett, M.L., and Eadie, T. 1981a. Potential mold growth, aflatoxin production and antimycotic activity of selected natural spices and herbs. J. Ass. Off. Anal. Chem. 64: 955.

Llewellyn, G.C., Dixon, E.C., Eadie, T., Dashek, W.V., and O'Rear, C.E. 1981b. Aflatoxin formation on whole and ground cumin and anise seeds. J. Am. Oil Chem. Soc. 58: 985 A.

Madhyashta, M.S. and Bhat, R.V. 1084. Aspergillus parasiticus growth and aflatoxin production on black and white pepper and the inhibitory action of their chemical constituents. Appl. Environ. Microbiol. 48: 376.

Mabrouk, S.S. and El-Shayeb, N.M.A. 1981. The effects of garlic on mycelial growth and aflatoxin formation by Aspergillus flavus. Chem. Microbiol. Technol. Lebensm. 7: 37.

Pal, N. and Kundu, A.K. 1972. Studies on Aspergillus sp. from Indian spices in relation to aflatoxin production. Sci. Cult. 38: 252.

Pons, W.A. Jr., Robertson, J.A., and Goldblatt, L.A. 1966. Objective fluorometric measurement of aflatoxins on TLC plates. J. Am. Oil Chem. Soc. 43: 665.

Pruthi, J.S. 1980. "Spices and Condiments." Advances in Food Research, Suppl. 4, p. 13. Academic Press, New York.

Ride, J.P. and Drysdale, R.B. 1972. A rapid method for the chemical estimation of filamentous fungi in plant tissue. Physiol. Plant Pathol. 2: 7.

Seenappa, M. and Kempton, A.G. 1980. Aspergillus growth and aflatoxin production on black pepper. Mycopathologia 70: 135.

Suzuki, J.I., Dainius, B.,and Kilbuck, J.N. 1973. A modified method for aflatoxin determination in spices. J. Food Sci. 38: 949.

Tiwari, R., Dikshit, R.P., Chandran, N.C. Saxena, A., Gupta, K.G., and Vadehra, D.D. 1983. Inhibition of growth and aflatoxin B_1 production of A. parasiticus by spice oils. J. Food Sci. Technol. 20: 131.

Udagawa, S. 1982. Detection and occurrence of mycotoxin producing fungi from imported foods in Japan. In "Control of the Microbial Contamination of Foods and Feeds in International Trade: Microbial Standards and Specifications," p. 229, (Ed.) Kurata, H. and Hesseltine, C.w. Saikon Publishing Co. Ltd., Tokyo.

Ms received 6/13/84; revised 11/7/84; accepted 11/11/84.

TIME-TEMP INDICATORS FOR FROZEN FOOD TRANSPORT... From page 371

Farquhar, J.W. 1977. Time-temperature indicators in monitoring the distribution of frozen foods. J. Food Quality 1(2): 119.

Hayakawa, K.I. and Wong, A.F. 1974. Performance of frozen food indicators subjected to time variable temperatures. ASHRAE J. 16(4): 44.

Heldman, D.R. and Lai, O.J. 1983. A model for prediction of shelf-life for frozen foods. IIR 16th International Congress of Refrig., Commission C2 Preprints, p. 427.

Kramer, A. and Farquhar, J.W. 1976. Testing of time-temperature indicating and defrost devices. Food Technol. 30(2): 50.

Labuza, T.P. 1982. "Shelf-life Dating of Foods." Food & Nutrition Press, Westport, CT.

Saguy, I. and Karel, M. 1980. Modeling of quality deterioration during food processing and storage. Food Technol. 34(2): 78.

Schoen, H.M. 1983. Thermal indicators for frozen foods. IIR 16th

International Congress of Refrig., Commission C2 Preprints, p. 427.

Schwimmer, S. and Ingraham, L.L. 1955. Temperature tolerance in frozen food processing effective temperatures in thermally fluctuating systems. Ind. & Eng. Chem. 47(6): 1149.

Van Arsdel, W.B., Copley, M.J., and Olson, R.L., Editors. 1959. "Quality and stability of frozen foods time-temperature tolerance and its significance." Wiley-Interscience, New York.

Ms received 7/13/84; revised 10/1/84; accepted 10/12/84.

SENSORY/COOKING QUALITY OF γ-IRRADIATED LEGUMES... From page 375

Snedecor, G.W. and Cochran, W.J. 1976. "Statistical Methods," p. 91. Iowa State University Press, Ames, IA.

Urbanyi, G. 1982. Investigation into the interaction of different properties in the course of sensory evaluation. Acta Aliment. 11: 233.

Vose, J.R. 1977. Functional characteristics of an intermediate amylose starch from smooth-seeded field peas compared with corn and wheat starch. Cereal Chem. 54: 1141.

Weiss, J. 1952. Chemical dosimetry using ferrous and ceric sulfates. Nucleonics 10: 28.

Williams, P.C., Nakoul, H., and Singh, K.B. 1983. Relationship between cooking time and some physical characteristics in chickpeas (Cicer arietinum L.). J. Sci. Food Agric. 34: 492.

Ms received 3/3/84; revised 10/3/84; accepted 10/5/84.

Effects of Gamma Irradiation Dose and Timing of Treatment after Harvest on the Storeability of Garlic Bulbs

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ABSTRACT

The effect of gamma irradiation dose and time of treatment after harvest on the storage of garlic bulbs was investigated. The effectiveness of irradiation for external sprout inhibition was not affected by the treatment time within 45 days after harvest. At 285 days after harvest, irradiation of 50 – 150 Gy caused about 6% less decrease in weight loss compared with the unirradiated group, and spoilage rates of the unirradiated and irradiated cloves were 100% and 17 – 20%, respectively. For the overall storageability of garlic bulbs, 75 Gy was shown to be the minimal optimum dose, and there was no apparent effect depending upon the time of irradiation treatment after harvest.

INTRODUCTION

LARGE QUANTITIES of the total garlic production in Korea spoil annually due to sprouting, which is usually followed by shrinkage, weight loss and decay. A number of investigators have studied the inhibition of sprouting in garlic bulbs by controlling the storage conditions (Kolev et al., 1971). However, it is a fact that the prolongation of the storage life of garlic bulbs until next harvest has many the storage life of garlic bulbs until next harvest has many problems with respect to the satisfactory sprout inhibition and additional loss reduction during storage. Since Sparrow and Christensen (1954) first stated that ^{60}Co gamma radiation improved the storage quality of potato tubers, many researchers have reported the advantage of gamma irradiation for the sprout inhibition of other root, bulb and tuber crops, with doses ranging from 20 – 150 Gy (Mekinney, 1971; Salem, 1974; Cho et al., 1983). Khan and Wahid (1978) proposed that 100 Gy irradiation followed by storage at 14 – 16°C was the optimum condition for storing potatoes, onions and garlic in a good state for more than five months in tropical countries. Lustre et al. (1982) found that the irradiated garlic bulbs with doses of 50 –120 Gy were marketable for 6 months when the unirradiated ones were already completely deteriorated. Thomas et al. (1975) also indicated that sprout inhibition by gamma irradiation was influenced by the physiological state of onions at the time of irradiation. In onions, it is now generally agreed that the sooner irradiation is used after harvest, the better will be the results and the lower the doses needed to inhibit sprouting (Mullins and Burr, 1961; Thomas et al., 1975). Hendel and Burr (1961), however, proposed that the effectiveness of irradiation for sprout control in potatoes was not affected by a delay between harvest and irradiation; garlic has not yet been investigated in this respect.

The present study was designed to determine whether irradiation dose and the time of treatment after harvest affected sprout and root control, weight loss, and spoilage of garlic bulbs during storage.

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MATERIALS & METHODS

Garlic

The garlic (*Allium sativum* L.) used was a Korean local late variety consisting of six to seven cloves and obtained from a chief producing district, Seosan.

Curing and irradiation

Garlic bulbs in bundles of 20 plants were hung on posts in a warehouse and cured in circulating air of 22 – 25°C and a relative humidity of 60 – 65% for 45 days after harvest. During curing, the garlic bulbs were irradiated with doses of 50, 75, 100, and 150 Gy by a 10 kilo curie ^{60}Co gamma irradiator (U.K., 250 Gy/hr) on the 15th, 30th, and 45th day after harvest, respectively, and divided into three groups.

Storage

The stems of the unirradiated and irradiated garlic samples were cut off 2 – 3 cm from the bulbs. The garlic bulbs were packaged individually in perforated plastic boxes (60 × 45 × 45 cm) and stored at 10 ± 5°C, and 70 – 80% relative humidity from the 45th day after harvest. The temperature and the relative humidity were controlled with an environmental controller (Keumsung Co., Ltd.).

Determination of moisture content

The moisture content of the cloves, skins and stems was determined according to the air oven method (Osborne and Voogt, 1981).

Measurement of sprouting and rooting

Sprouting and rooting rates were measured bi-monthly and expressed as the percentage of sprouted or rooted cloves per 100 cloves examined. Sprouting and rooting of stored garlic bulbs were measured as 1 mm lengths of sprout or root development from the cloves.

Measurement of weight change

Two kilograms of garlic bulbs from each group were weighed at bi-monthly intervals, and the cumulative percentage of weight loss was calculated.

Evaluation of spoilage

The spoilage of garlic bulbs was determined at 285 days after harvest and expressed as the percentage of spoiled cloves per 100 cloves. Evaluation criteria were based on the observation of any symptom among the external sprouting, rooting, shrinkage, discoloration, disease, and hollow cloves.

Analysis of data

The results were analyzed statistically using the T-test and analysis of variance. All figures reported here represent the average of triplicate experiments.

RESULTS & DISCUSSION

Moisture content of garlic bulbs during curing

During the drying of post-harvest garlic bulbs, the moisture content of cloves was relatively constant, while that of the skins and stems rapidly decreased. To standardize the drying of garlic bulbs before placing them in storage, each treatment group was cured collectively for 45 days, al-

though the moisture content of the bulbs differed at the three periods of irradiation. After curing, moisture contents of the cloves, skins and stems were 64.0%, 14.9% and 15.3%, respectively (Table 1).

Sprout and root inhibition of garlic bulbs during storage

The effects on sprout and root inhibition of the garlic bulbs irradiated with several doses on the 15th, 30th and 45th days after harvest are given in Table 2. The sprout leaves of the unirradiated bulbs emerged partially from the cloves at about 165 days after harvest, whereas all irradiated bulbs showed complete inhibition of external sprouting up to 285 days after harvest. El-Oksh et al. (1971) observed that sprouts in the gamma irradiated or maleic hydrazide-treated garlic cloves remained internal for 268 days when stored at room temperature, while those of the unirradiated bulbs were produced from cloves after 106 days storage. Mathur (1963) also indicated that garlic bulbs irradiated with 50 Gy within 1 month after harvest could be stored in a sound state for 7 months at $11 - 12°C$ and a relative humidity of 85 – 95%, as against about 2 months for the unirradiated samples. The results observed agree well with the above reports.

At the end of the storage period the rooting rates of the unirradiated and 50 Gy groups were 100% and 23%, respectively, while no root growth occurred in the other irradiated bulbs during the entire storage period, showing a similar tendency among the three groups. Similar results were obtained by other investigators (Kwon, 1983; El-Oksh et al., 1971).

Weight change of garlic bulbs during storage

The physiological losses in weight were mainly due to transpiration and respiration (Hendel and Burr, 1961; Mathur, 1963). The cumulative percentage of weight loss was lower in the irradiated bulbs than in the unirradiated ones during storage ($P < 0.05$) (Table 3). Especially, at 285 days after harvest, gamma irradiation caused about 6% less decrease in weight loss as compared with the unirradiated bulbs. This result confirms the observations of El-Oksh et al. (1971), Abdel-Al (1967) and Kwon (1983) with garlic and Cho et al. (1983) and Lustre et al. (1982) with onions. However, there was no significant difference among the irradiated groups in the time of the irradiation treatment.

Spoilage of garlic bulbs

At 285 days after harvest, the effects of irradiation dose and time of treatment on the spoilage of garlic cloves is shown in Fig. 1. Spoilage rates based on the external sprouting, rooting, shrinkage, discoloration, disease, and hollow cloves were 100% in the unirradiated cloves and 17 – 20% in the irradiated ones. Among the irradiated groups, 75 and 100 Gy irradiated cloves were somewhat superior to those of 50 and 150 Gy; however, some shrunken or dried sprout leaves and empty cavities were observed in the 100 and 150 Gy irradiated cloves. Empty cavities

Table 1—Moisture content of post-harvest garlic bulbs during curing at 22 - 25°C[a]

Parts	Curing period (days after harvest)			
	0	15[b]	30[b]	45[b]
Cloves	66.6	64.5	64.0	64.0
Skins	74.1	47.4	22.1	14.9
Stems	76.0	50.0	23.8	15.3

[a] Each value is the average content (%) for 10 bulbs.
[b] Lapsed time between harvest and irradiation.

Table 2—Effect of the irradiation dose and timing of treatment after harvest on the sprouting and rooting of garlic bulbs during storage at 10 ± 5°C[a]

Storage period (days after harvest)	Irradiation timing (days after harvest)														
	15					30					45				
	0[b]	50	75	100	150	0[b]	50	75	100	150	0[b]	50	75	100	150
105	0 (42)	0 (0)	0 (0)	0 (0)	0 (0)	0 (42)	0 (0)	0 (0)	0 (0)	0 (0)	0 (42)	0 (18)	0 (0)	0 (0)	0 (0)
165	21 (100)	0 (18)	0 (0)	0 (0)	0 (0)	21 (100)	0 (18)	0 (0)	0 (0)	0 (0)	21 (100)	0 (23)	0 (0)	0 (0)	0 (0)
225	96 (100)	0 (18)	0 (0)	0 (0)	0 (0)	96 (100)	0 (23)	0 (0)	0 (0)	0 (0)	96 (100)	0 (23)	0 (0)	0 (0)	0 (0)
285	100 (100)	0 (23)	0 (0)	0 (0)	0 (0)	100 (100)	0 (23)	0 (0)	0 (0)	0 (0)	100 (100)	0 (23)	0 (0)	0 (0)	0 (0)

[a] Sprouting and rooting are expressed as the percentage of sprouted or rooted cloves per 100 cloves examined and number in parenthesis designates rooting rate.
[b] Irradiation dose (Gy).

Table 3—Effect of the irradiation dose and timing of treatment after harvest on the weight change of garlic bulbs during storage at 10 ± 5°C[a]

Storage period (days after harvest)	Irradiation timing (days after harvest)														
	15					30					45				
	0[b]	50	75	100	150	0[b]	50	75	100	150	0[b]	50	75	100	150
105	14.5	13.4	13.3	13.8	13.9	14.5	13.5	13.0	13.9	13.5	14.5	13.6	13.5	14.1	13.8
165	25.8	22.9	22.2	22.0	23.1	25.8	23.1	22.2	22.1	22.9	25.8	23.5	22.9	22.5	23.0
225	32.2	30.8	29.1	27.3	29.4	32.2	29.9	29.2	27.8	29.0	32.2	30.5	29.4	28.1	29.6
285	37.0	32.2	31.9	29.6	31.9	37.0	32.0	32.0	29.4	31.2	37.0	32.9	32.3	30.0	31.5

[a] Weight change is expressed as the percentage of weight loss per initial weight and each value is the average for triplicate experiments. $P < 0.05$ between unirradiated and irradiated groups and $P > 0.05$ among the treatment timing of irradiation.
[b] Irradiation dose (Gy).

that developed in garlic cloves during storage were one of the most important factors influencing the quality of garlic bulbs (El-Oksh et al., 1971).

Also, it was shown that the spoilage of garlic bulbs was slightly affected by the time of the irradiation treatment within 45 days after harvest.

Considering the effect of irradiation on the storageability of garlic bulbs, 75 Gy was shown to be the minimal optimum dose. There was no apparent effect from the time of the irradiation treatment after harvest on the storage of the bulbs.

REFERENCES

Abdel-Al, Z.E. 1967. The possibility of using gamma radiation to improve the keeping quality of Egyptian garlic. Alex. J. Agric. Res. 15: 381.

Cho, H.O., Kwon, J.H., Byun, M.W., and Yang, H.S. 1983. Batch scale storage of sprouting foods by irradiation combined with natural low temperature. 1. Storage of onions. J. Korean Agric. Chem. Soc. 26: 82.

El-Oksh, I.I., Abdel-Kader, A.S., Wally, Y.A., and El-Kolly, A.F. 1971. Comparative effects of gamma irradiation and maleic hydrazide on storage of garlic. J. Am. Soc. Hort. Sci. 96: 637.

Hendel, C.E. and Burr, H.K. 1961. Treatment of potatoes with gamma rays; Effects of delay between harvest and irradiation. Food Technol. 15: 218.

Khan, I. and Wahid, M. 1978. Feasibility of radiation preservation of potatoes, onions and garlic in Pakistan. In "Food Preservation by Irradiation," Vol. 1, p. 63, IAEA, Vienna.

Kolev, N., Poppov, I., and Doganova, L. 1959. Changes in weight, chemical composition and activity of an enzyme system of garlic in relation to storage conditions. Arch. Gartenbau. 7: 52.

Kwon, J.H. 1983. Effects of irradiation on the storeability of garlic and its pungent flavor components. Ph.D. thesis, Kyungpook National Univ., Korea.

Lustre, A.O., Roncal, R.A., Villaruel, F.G., Ang. L., Singson, C.C., Carmona, C.L., and DeGuzman, Z.M. 1982. The technological feasibility of gamma radiation for the extended commercial storage of agriculture crops, onion and garlic. Food Irradiation for Developing Countries in Asia and Pacific, p. 127, IAEA, Vienna.

Mathur, P.B. 1963. Extension of storage life of garlic bulbs by γ-irradiation. J. Appl. Rad. Isotopes. 14: 625.

Mekinney, F.E. 1971. Potato-sprout inhibition by γ-irradiation. 1. Isotopes & Radiation Technol. 8: 87.

Mullins, W.R. and Burr, H.K. 1961. Treatment of onions with gamma rays: Effects of delay between harvest and irradiation. Food Technol. 15: 178.

Osborne, D.R. and Voogt, P. 1981. "The Analysis of Nutrients in Foods," p. 107. Academic Press, London.

Salem, S.A. 1974. Effect of gamma radiation on the storage of onions used in the dehydration industry. J. Sci. Food Agric. 25: 257.

Sparrow, A.H. and Christensen, E. 1954. Improving storage quality of potato tubers after exposure to Co-60 gammas. Nucleonics 12: 16.

Thomas, P., Srirangarajan, A.N., and Limaye, S.P. 1975. Studies on sprout inhibition of onions by gamma irradiation. 1. Influence
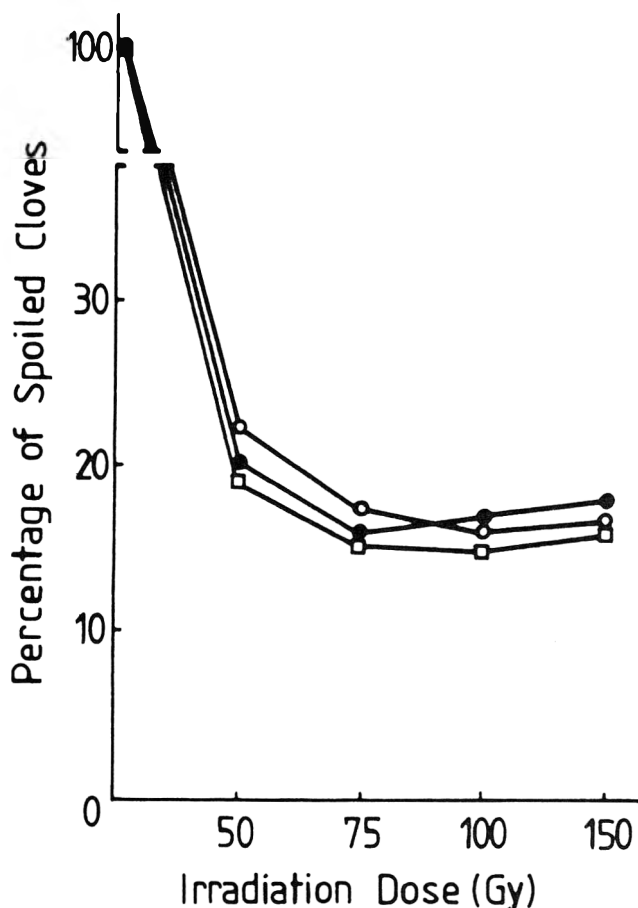
Fig. 1—Effect of the irradiation dose and time of treatment after harvest on the spoilage of garlic bulbs at 285 days after harvest. Each value is the mean of triplicate experiments: Irradiation time after harvest, •, 15th day; □, 30th day; ○, 45th day.

of time interval between harvest and irradiation, radiation dose and environmental conditions on sprouting. Radiation Botany 15: 215.

Amylographic viscosity values for rice flour from the various toasting treatments indicate (limited amount of flour resulted in single values, therefore, significance could not be determined) that the pasting temperature of the flour was elevated by toasting but was not further increased with prolonged toasting (Table 5). This indicates that the rate of swelling decreased since the dry weight of the flour was constant. The viscosity of the paste at 92.5°C further indicates that toasting influenced the rate of swelling and also that as toasting time was extended the swelling rate further decreased. The effect of toasting on the swelling rate continued to be apparent after 15 min at 92.5°C. Hot paste stability declined (viscosity decreased during the 15 min holding at 92.5°C) for the nontoasted flour increased for all toasted flours. The rate of change in hot paste viscosity tended to increase with increased severity of toasting. The nontoasted flour reached a much higher viscosity after cooling to 25°C but the rate of viscosity increase (viscosity after cooling to 25°C divided by viscosity before cooling) appeared to be nearly the same for all degrees of toasting (2.2 − 2.5). The rate of retrogradation seemingly was unaffected by the toasting treatments.

REFERENCES

Bhattacharya, K.R. 1979. Tests for parboiled rice. In "Proceedings of the Workshop on Chemical Aspects of Rice Grain Quality." Los Banos, Laguna, Philippines.

Fellers, D.A., Mossman, A.P., and Suzuki, H. 1983. Rice stickiness. 2. Application of an Instron method to make varietal comparisons and to study modification of milled rice by hot-air treatment. Cereal Chem. 160: 292.

Knight, E., Okoro, C., and Okaka, J.C. 1982. Taste and biological response of toasted wheat and soy-wheat breads. Nutr. Rep. Int. 26: 827.

Noble, A.C. 1975. Instrumental analysis of the sensory properties of food. Food Technol. 29(12): 56.

Sharp, R.N., Sharp, C.Q., and Kattan, A.A. 1981. A new method for thermally processed canned rice. Food Technol. 35(5): 75.

Sharp, R.N., Kattan, A.A., Sharp, C.Q., and Collins, J.A. 1982. Safety and sensory evaluation of canned rice. J. Food Sci. 47: 1123.

Sharp, R.N., Kattan, A.A., and Sharp, C.Q. 1984. The effects of toasting on rice cooking quality. Ark. Farm Res. 33(4): 11.

Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics." McGraw-Hill Book Co., Inc., New York, NY.

Trant, A.S., Pangborn, R.M., and Little, A.C. 1981. Potential fallacy of correlating hedonic responses with physical and chemical measurements. J. Food Sci. 46: 583.

Use of an Edible Film to Maintain Water Vapor Gradients in Foods

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─────────ABSTRACT─────────

An edible, bilayer film consisting of a layer of stearic-palmitic acid and a layer of hydroxypropyl methylcellulose was situated between two food components of markedly different water activities to determine the film's ability to retard equalization of water activity. Tomato paste or salted tomato paste was used as the high-moisture food and ground crackers were used as the low-moisture food. Compared to a filter paper control, the bilayer film substantially slowed transfer of water from the salted tomato paste to the crackers during 14 days at 25°C and 21 days at 5°C. During 70 days at −20°C, the film essentially stopped the transfer of water from tomato paste to the crackers.

INTRODUCTION

MOISTURE TRANSFER in finished food products frequently leads to deleterious changes in product quality. The availability of an edible food coating which prevents moisture exchange between components of food mixtures would be highly desirable.

Analysis of the moisture barrier properties of edible food coatings applied to food products has been limited to measurements of moisture change (moisture gain or loss) and quality change (texture, color and flavor) (Allen et al., 1963a, b; Bauer et al., 1968; Cole, 1969; Cosler, 1957; D'Atri et al., 1980; Earle, 1968; Earle and McKee, 1976; Earle and Snyder, 1966; Hamdy and White, 1969, Lazarus et al., 1976; McKee, 1978, Shaw et al., 1980; Shea, 1970; Silva et al., 1981; Stemmler and Stemmler, 1974; Ukai et al., 1976; Watters and Brekke, 1959, 1961; Werbin et al., 1970; Williams et al., 1978). These tests are useful for the qualitative screening of films of various compositions; however, they provide inexact indications of the ability of an edible film to maintain a specific a_w gradient in a food product.

To accurately assess the effectiveness of a moisture impermeable film, the moisture content-water activity properties of the system must be known and considered. Moisture transfer will occur in a food when moisture vapor pressure (a_w) gradients exist, regardless of the moisture contents of the individual food components (Salwin and Slawson, 1959). In contrast, moisture content gradients can be maintained in food products provided the water activities of the components are the same (Bone, 1969). Knowledge of the moisture content-water activity state of food components is also necessary to accurately interpret the importance of moisture changes occurring in food products.

An edible film composed of a lipid layer and a polysaccharide layer was found to be an effective moisture vapor barrier with model systems (Kamper and Fennema, 1984). The objective of this study is to test the ability of this film to maintain a water activity gradient existing between two food components.

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MATERIALS & METHODS

Sample preparation

A bicomponent food consisting of saltine crackers (Nabisco) and tomato paste (Contadina) was selected for study. Although this combination does not represent a food item of commerce, it was convenient to use and provided a water activity gradient and physical properties similar to those exhibited by fabricated food materials in which moist and dry components are in contact and are expected to retain distinctly different physical properties during storage.

The crackers were pulverized in a blender. For above-freezing studies, the a_w of the tomato paste was lowered by the addition of NaCl (15%) to water activities of 0.86 and 0.82 at 25°C and 5°C, respectively. This was done since preliminary studies showed that the film would soften and/or disintegrate when exposed to unmodified tomato paste (a_w above 0.95) at these temperatures. The tomato paste was not modified when used at subfreezing temperatures since the a_w at −20°C (0.82; Fennema and Berny, 1974) was sufficiently low to avoid this problem.

Film

The edible film used in this study was the stearic acid-palmitic acid emulsion film (C_{18}-C_{16} E-Film) described by Kamper and Fennema (1984). The average fatty acid concentration was 0.85 mg/cm^2 film with a range of 0.80 – 0.90 mg/cm^2 film. Thickness of the films varied from 0.03 – 0.04 mm (1.3 – 1.7 mils).

Storage conditions

The food samples were placed in 39.3 cm^3 (6.3 cm o.d., 5.0 cm i.d., and 2.0 cm deep) cups with flat lips. Two cups, one containing ground saltine crackers and another containing tomato paste were needed to prepare one sample with a high moisture-high a_w fraction (tomato paste) and a low moisture-low a_w fraction (crackers) (Fig. 1).

The sets of cups had either the C_{18}-C_{16} E-Film or a filter paper (control) separating the two food components. The filter paper, (Whatman 54 hardened, fast retention; Whatman Laboratory Products, Inc., Clifton, NJ) offered negligible resistance to moisture transfer. The filter paper was added simply to prevent the ground crackers from adhering to the tomato paste, thus allowing the two components to be easily separated and analyzed after storage. The lipid side of the C_{18}-C_{16} E-Film faced the tomato paste. The two cups, separated by either a filter or a film, were combined and sealed with a blend of microcrystalline and paraffin wax.
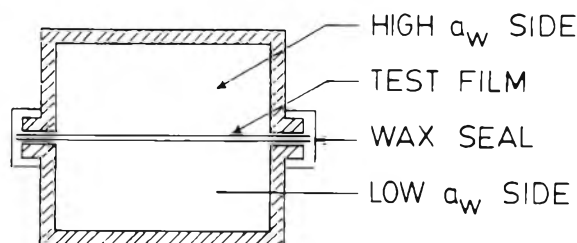

Fig. 1—Schematic diagram of container for sample storage. High a_w-high moisture side (tomato paste); low a_w-low moisture side (crackers); test film, either moisture barrier (C_{18}-C_{16} E-Film) or control (filter paper); wax seal (microcrystalline-paraffin wax blend).

The cups of tomato paste and crackers intended for frozen storage, were stored separately for 16 hr at −20°C to freeze the tomato paste and thus lower the a_w of the tomato paste to 0.82 at −20°C (Fennema and Berny, 1974). The film was then applied and the cups were assembled and sealed. The bicomponent food samples were stored at 25°C ± 2°C, 5°C ± 2°C or −20°C ± 2°C.

Sampling

Sampling times were chosen based on the progression of moisture transfer between the two components. At suitable times, dishes were removed from storage, the wax seals broken, the components separated and analyzed for moisture content. All test variables and analyses were performed in duplicate.

Moisture transfer analysis

Moisture contents for tomato paste and saltine crackers were determined by the vacuum oven method (5 hr, 100°C and 30 inches Hg vacuum, AOAC, 1975).

Water activity values of the crackers were obtained from a moisture sorption isotherm for saltine crackers. The moisture sorption isotherm was prepared by allowing 5g samples of ground saltine crackers to equilibrate in vacuumized desiccators at 25°C in the presence of saturated salt solutions of known a_w. The salt solutions used were: LiCl ($a_w = 0.11$) CH_3COOK ($a_w = 0.23$), $MgCl_2$ ($a_w = 0.33$), K_2CO_3 ($a_w = 0.43$), NaBr ($a_w = 0.58$), $NaNO_2$ ($a_w = 0.65$), NaCl ($a_w = 0.75$), KCl ($a_w = 0.85$) and $BaCl_2$ ($a_w = 0.90$) (Rockland, 1960; Wink and Sears, 1950).

Water activity values of the tomato paste were determined using a Beckman Sina-Scope, Model SJT-B, humidity instrument (range 50 – 95% RH) calibrated with saturated salt solutions.

Because a_w is temperature dependent, the a_w values of the samples at 5°C and −20°C were corrected for the temperature difference between the measurement temperature (25°C) and the actual storage temperature (5°C or −20°C). The a_w of tomato paste (5°C), which decreased only slightly during storage with crackers,

was simply corrected using a temperature coefficient of 0.002 (a_w change per degree centigrade) (Fennema, 1981).

Since temperature coefficients for correcting water activity vary with the moisture content of the sample, a single temperature coefficient could not be used to correct the a_w of crackers at 5°C and −20°C because the moisture content of these samples increased significantly during storage. Instead, published isotherms for native potato starch, 2.5 – 20°C (van den Berg and Leniger, 1978) and bovine muscle, −10°C to −20°C (MacKenzie, 1975) were used to estimate correction factors for the a_w of crackers stored at 5°C and −20°C. Although these correction factors are not highly accurate, they are preferable to using uncorrected a_w values, or a_w values corrected with a single temperature coefficient. Furthermore, good precision in determining a_w values was of primary concern in this study, and a high level of accuracy was of secondary importance.

RESULTS & DISCUSSION

CRACKERS AND TOMATO PASTE were selected as the components for the bicomponent food because of their large difference in a_w, their similarity to existing products (for example, pizza crust/pizza sauce) and their ease of use. The initial a_w gradient between the two components was 0.76 at 25°C, 0.75 at 5°C and 0.78 at −20°C. These a_w gradients are somewhat larger than typically found in nonhomogeneous foods (Table 1), and were selected to evaluate the barrier properties of the edible film under severe but realistic conditions.

Moisture transfer, expressed in terms of a_w, from tomato paste to crackers, with the components separated by either the C_{18}-C_{16} E-Film or a filter paper control, are shown for three temperatures in Fig. 2, 3 and 4.

The cracker component of the bicomponent food exhibited greater changes in water content and water activity than the tomato paste component (all storage temperatures and films) because so little water was originally present in the crackers. The a_w of crackers stored with the filter paper control increased rapidly at 25°C and 5°C, attaining vapor pressure equilibrium with the tomato paste after about 10 days and 28 days, respectively (Fig. 2 and 3). However, at vapor pressure equilibrium, a water concentration gradient still existed. Establishment of equilibrium at −20°C (filter paper control) was of course much slower than at higher temperatures (Fig. 4).

The C_{18}-C_{16} E-Film, at both 25°C and 5°C, substantially slowed the rate of moisture transfer as compared to the filter paper controls (Fig. 2 and 3). At −20°C, the edible barrier essentially stopped the transfer of moisture during the

Table 1—Water activity gradients in nonhomogeneous food products

High a_w component			Low a_w component	
Fish & Poultry	0.98 – 1.00	vs	Breading	0.67 – 0.87
Baked cake	0.90 – 0.94	vs	Cake Icing	0.76 – 0.84
Dried fruit	0.55 – 0.80	vs	Cereal	0.10 – 0.20
Refrigerated biscuit dough	0.94	vs	Pastry filling	0.60 – 0.70
Bakery goods	0.90 – 0.95	vs	Nuts	0.20 – 0.30
Dehydrated beef soup & gravy base	0.34	vs	Dehydrated beef	0.01 – 0.04

Fig. 2—Moisture transfer, expressed in terms of a_w at 25°C, from salted tomato paste to saltine crackers, with the two components separated by either an edible film ("w-film") or a filter paper ("w/o film"). Edible film: C_{18}-C_{16} E-Film, 0.85 mg lipid/cm² film, thickness 1.3 – 1.7 mils. Variability between the duplicate samples is shown only when variability exceeds the size of the symbol.
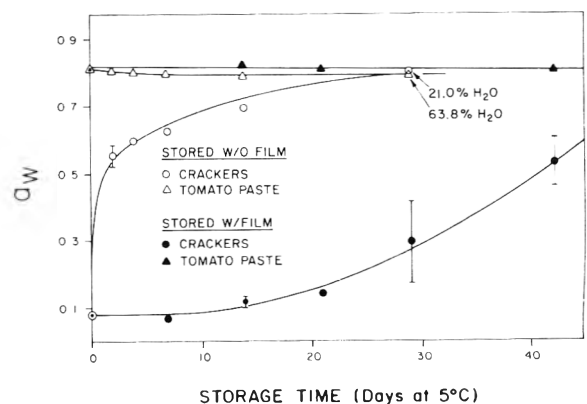
Fig. 3—Moisture transfer, expressed in terms of a_w at 5°C, from salted tomato paste to saltine crackers, with the two components separated by either an edible film ("w/film") or a filter paper ("w/o film"). Edible film: C_{18}-C_{16} E-Film, 0.85 mg lipid/cm² film, thickness 1.3 – 1.7 mils. Variability between the duplicate samples is shown only when variability exceeds the size of the symbol.

70 day storage period (Fig. 4). The position of the tomato paste (top or bottom) did not influence the results (data not shown).

The cracker component of some samples stored at $25°C$ or $5°C$ in the presence of the edible film (Fig. 2 and 3) exhibited relatively large variability in a_w. This variability probably resulted from flaws in the film or in the wax used to seal the test cups. The increasing rate of moisture transfer across the C_{18}-C_{16} E-Films after about 5 wk at $25°C$ or $5°C$ (exhibited by changes in the a_w of the cracker component) suggests that alterations occurred in film properties. All films were examined visually after storage and although evidence of discontinuities was not apparent, all films after storage for at least 5 wk, were more elastic, indicating that hydration of the film had occurred. If so, an increase in permeability to water vapor would be predicted (Karel, 1975).

Critical values of water activity (a_c), that is, values above which products become unacceptable, have been established for many moisture sensitive products. Using an a_c value of 0.39 for saltine crackers (Katz and Labuza, 1981), the C_{18}-C_{16} E-Film can be evaluated for its ability to extend the shelf life of the bicomponent food product. Crackers stored with the filter paper control reached the a_c within 1 day at $25°C$ and $5°C$ and within about 3 wk at $-20°C$. In comparison, the crackers separated from the tomato paste by the C_{18}-C_{16} E-Film reached the a_c in approximately 3 wk at $25°C$, in about 5 wk at $5°C$ and were far from attaining this value after 10 wk at $-20°C$.

It should be noted that the water vapor differentials employed in this study were greater than those which would be encountered in many commercial situations (Table 1), thus the C_{18}-C_{16} E-Film would be expected to be even more effective than the results in Fig. 2, 3 and 4 would indicate.

The application of this film, as presently formulated, is limited to food products with water activites of 0.90 or below when above-freezing temperatures are employed. However, foods with water activities greater than 0.90 (when measured above freezing) may be used if the film is applied after the food has been frozen to a temperature of $-10°C$ or lower.

Film of the type studied here would appear to have a good potential for commercial success as edible moisture barriers. Further tests are necessary to determine film durability under commercial situations.

REFERENCES

Allen, L., Nelson, A.I., Steinberg, M.P., and McGill, J.N. 1963a. Edible corn-carbohydrate food coatings. 1. Development and physical testing of a starch-algin coating. Food Technol. 17: 1437.

Allen, L., Nelson, A.I., Steinberg, M.P., and McGill, J.N. 1963b. Edible corn-carbohydrate food coatings. 2. Evaluation on fresh meat products. Food Technol. 17: 1442.

AOAC. 1975. "Official Methods of Analysis," 12th ed., (Ed.) Horwitz, W. Assn. Offic. Anal. Chemists, Washington, DC.

Bauer, C.D., Neumer, G.L., and Pinkalla, H.A. 1968. Method for preparing a coated meat product. U.S. patent 3,406,081.

Bone, D.P. 1969. Water activity — its chemistry and application. Food Prod. Devel. August, p. 81.

Cole, M. 1969. Method for coating dehydrated food. U.S. patent 3,479,191.

Cosler, H.B. 1957. Method of producing zein-coated confectionery. U.S. patent 2,791,509.

D'Atri, J.J., Swidler, R., Colwell, J.J., and Parks, T.R. 1980. Fat based preservative coating and method for preserving foods. U.S. patent 4,207,347.

Earle, R.D. 1968. Method of preserving foods by coating same. U.S. patent 3,395,024.

Earle, R.D. and McKee, D.H. 1976. Process for treating fresh meats. U.S. patent 3,991,218.

Earle, R.D. and Snyder, C.E. 1966. Method of preparing frozen seafood. U.S. patent 3,255,021.

Fennema, O. 1981. Water activity at subfreezing temperatures. In "Water Activity: Influences on Food Quality," (Ed.) Rockland, L.B. and Stewart, G.F. Academic Press, Inc., New York.
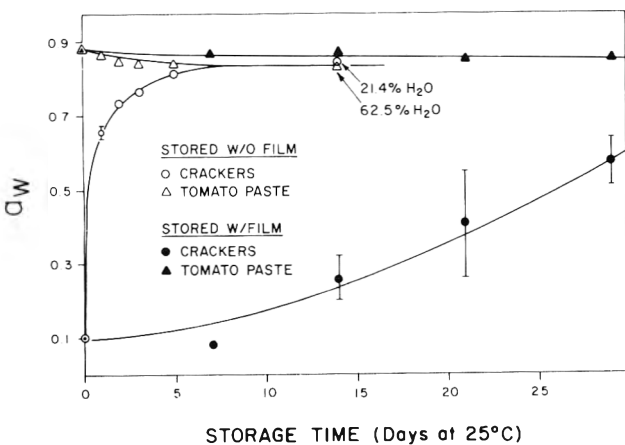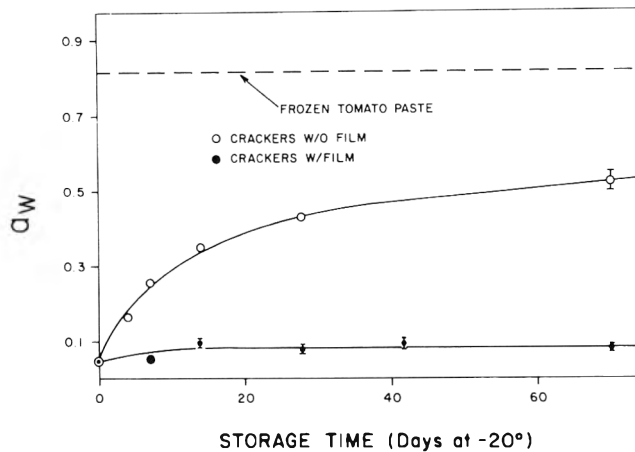
Fig. 4—Moisture transfer, expressed in terms of a_w at $-20°C$, from tomato paste to saltine crackers, with the two components separated by either an edible ("w/film") film or a filter paper ("w/o film"). Edible film: C_{18}-C_{16} E-Film, 0.85 mg lipid/cm^2 film, thickness 1.3 – 1.7 mils. Variability between the duplicate samples is shown only when variability exceeds the size of the symbol.

Fennema, O. and Berny, L.A. 1974. Equilibrium vapor pressure and water activity of food at subfreezing temperatures. Proc. IV Int. Congress Food Sci. & Technol., Vol. 2, p. 27.

Hamdy, M.M. and White, H.S. 1969. Edible coating compositions. U.S. patent 3,471,304.

Kamper, S.L. and Fennema, O. 1984. Water vapor permeability of edible bilayer films. J. Food Sci. 49(6): 1478.

Karel, M. 1975. Protective packaging of food. In "Principles of Food Science, Part II, Physical Principles of Food Preservation." (Ed.) Karel, M., Fennema, O.R., and Lund, D.B. Marcel Dekker, Inc., New York.

Katz, E.E. and Labuza, T.P. 1981. Effect of water activity on the sensory crispness and mechanical deformation of snack food products. J. Food Sci. 46: 403.

Lazarus, C.R., West, R.L., Oblinger, J.L., and Palmer, A.Z. 1976. Evaluation of a calcium alginate coating and a protective plastic wrapping for the control of lamb carcass shrinkage. J. Food Sci. 41: 639.

MacKenzie, A.P. 1975. The physicochemical environment during the freezing and thawing of biological materials. In "Water Relations in Foods," (Ed.) Duckworth, R.B. Academic Press, London.

McKee, D.H. 1978. Coating pizza products. U.S. patent 4,066,796.

Rockland, L.B. 1960. Saturated salt solutions for static control of relative humidity between 5° and 40°C. Anal. Chem. 32: 1375.

Salwin, H. and Slawson, V. 1959. Moisture transfer in combinations of dehydrated foods. Food Technol. 13: 715.

Shaw, C.P., Secrist, J.L., and Tuomy, J.M. 1980. Method of extending the storage life in the frozen state of precooked foods and product produced. U.S. patent 4,196,219.

Shea, R.A. 1970. Fruit containing baking mixes. U.S. patent 3,516,836.

Silva, R., Ash, D.J., and Scheible, C.E. 1981. Process for applying a moisture barrier coating to baked and fried food products. U.S. patent 4,293,572.

Stemmler, M. and Stemmler, H. 1974. Method for preserving freshly slaughtered meat. U.S. patent 3,851,077.

Ukai, N., Ishibashi, S., Tsutsumi, T., and Marakami, K. 1976. Preservation of agricultural products. U.S' patent 3,997,674.

van den Berg, C. and Leniger, H.A. 1978. The water activity of foods. In "Food Technology at the Agricultural University of Wageningen," (Ed.) Bruin, S.H. Veenman and Zonen, B.V., Wageningen, the Netherlands.

Watters, G.G. and Brekke, J.E. 1959. Coating of raisins and other foods. U.S. patent 2,909,435.

Watters, G.G. and Brekke, J.E. 1961. Stabilized raisins for dry cereal products. Food Technol. 15(5): 236.

Werbin, S., Rubenstein, I.H., and Weinstein, D. 1970. Method of water-proofing baked goods. U.S. patent, 3,526,515.

Williams, S.K., Oblinger, J.L., and West, R.L. 1978. Evaluation of a calcium alginate film for use on beef cuts. J. Food Sci. 43: 292.

Wink, W.A. and Sears, G.R. 1950. Equilibrium relative humidities above saturated salt solutions at various temperatures. TAPPI 33(9): 96A.

Effect of Temperature on the Moisture Sorption Isotherms and Water Activity Shift of Two Dehydrated Foods

T. P. LABUZA, A. KAANANE, and J. Y. CHEN

ABSTRACT

The water activity (a_w) of eight salt solutions was determined at three temperatures (25, 30, 45°C) using a pressure transducer-vapor pressure manometer. The a_ws of the salts showed a decrease with increasing temperature, which was explained with the help of a thermodynamic equation. This is opposite to the increase in a_w with increase in temperature for foods. Moisture sorption data for fish flour and cornmeal were obtained at 25-65°C. The Guggenheim-Anderson-deBoer model was evaluated and shown to be comparable to the Brunauer-Emmett-Teller model for prediction of the monolayer. Product was equilibrated at different a_ws at 25°C then subsequently shifted to 30°C and 45°C in a sealed chamber. The resultant a_w change, measured on the Kaymont-Rotronics, was predictable from the isotherm at each temperature using the Clausius Clapeyron relationship.

INTRODUCTION

AN IMPORTANT FACTOR affecting the stability of dehydrated foods is water activity (a_w). Both chemical reaction rates and microbial activity are directly controlled by a_w (Scott, 1957; Labuza, 1970; Troller and Christian, 1978). Salwin (1959) and Labuza (1970) showed that most deteriorative reactions in food systems have the lowest rate at the Brunauer-Emmett-Teller (BET) monolayer (Brunauer et al., 1938) which usually corresponds to the 0.2-0.4 a_w range. An increase in a_w beyond this region will induce an increase in the reaction rate generally by a factor of 50-100% for each 0.1 a_w change. This shift could happen under two different conditions. First, if a product made at some stable a_w is sealed in moisture permeable packaging material and held at high external humidity, the product will gain moisture from the atmosphere. The a_w will obviously increase, resulting in decreased shelf life. The second condition occurs if the food is sealed in impermeable packaging material and then subjected to an upward temperature shift. Fig. 1 shows two isotherms, one at 25°C and the other at 45°C for the same food, indicating that at any constant moisture content, a_w increases with increasing temperature. The increase in a_w should be predictable from the sorption isotherm at the higher temperature if this is available. Alternatively, some other method of measuring the a_w shift at the higher temperature would be required.

Labuza (1968), Loncin (1980) and Iglesias and Chirife (1976b) have shown that the Clausius Clapeyron equation can be applied to predict the isotherm a_w value at any temperature if the corresponding heat of sorption is known at constant moisture content. The equation is:

$$\ln \frac{a_2}{a_1} = \frac{Q_s}{R}\left[\frac{1}{T_1} - \frac{1}{T_2}\right] \qquad (1)$$

where: a_1, a_2 are water activities at temperature T_1 and T_2

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(°K); R = gas constant (1.987 cal/mole °K); Q_s = excess heat of sorption (cal/mole).

To determine Q_s, the sorption isotherm must be measured at a minimum of two temperatures; however, more temperatures in the range of study will give a better estimate. This is because several assumptions are made in applying the Clausius Clapeyron equation. First, the heat of vaporization of pure water (ΔH_v) and the excess heat of sorption (Q_s) do not change with temperature. Secondly, the equation applies only when the moisture content of the system remains constant. These assumptions could be met for a pure system at low temperature; however, for complex systems like food, some irreversible changes can occur in the water blinding properties of the system, especially with extrapolation to very high temperatures such as during extrusion processing. From two moisture isotherms determined at least at 10°C apart, the a_w at any other temperature should be predictable from Eq. (1) if the assumptions are correct.

At room temperature, water sorption isotherms are known for many foods (Iglesias and Chirife, 1982). However, according to a recent review by Bruin and Luyben (1980), few investigations have been conducted at higher temperatures. Graciala et al. (1982) obtained sorption isotherms for apples at 20-60°C using the desiccator method (Gal, 1981). The typical shift of the isotherm seen in Fig. 1 as temperature increases was found. One problem in the Graciala study was that salt solutions were used to maintain the desired a_w. The a_w values used for the sorption isotherm plotted at high temperatures were the salt solution a_w values obtained at 25°C, i.e., no correction was made for the shift in a_w of the salt solutions with temperature, although for some salts such as NaCl the response is flat. For others, the a_w shift would introduce error in the value of Q_s and any subsequent predictions.
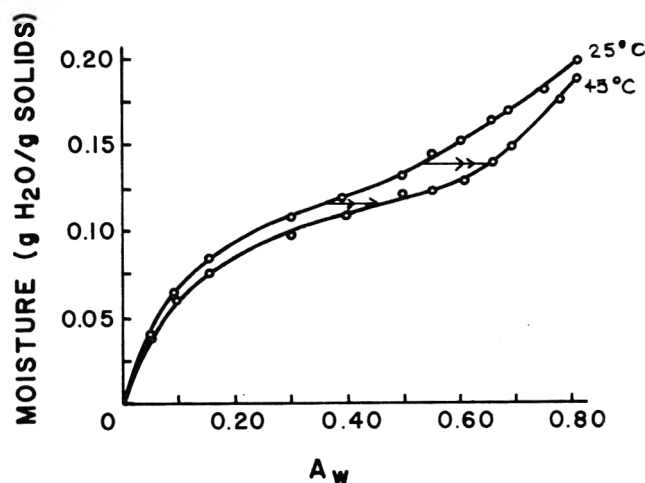

Fig. 1—Theoretical moisture sorption isotherms for a food at 25°C and 45°C showing the potential a_w shift if a product were transferred from 25°C to 45°C at constant moisture content in a sealed package.

The most frequently used methods for measuring a_w, such as gravimetric or hygrometric techniques, require standard reference sources for each a_w value. These references are needed for maintaining a constant relative humidity in the space around the sample for sorption isotherm determination or for proper calibration of electric hygrometers used to measure the a_w. Generally, saturated salt solutions have been used by most workers (Carr and Harris, 1949). Much literature data and several reviews are available giving the a_w of salt solutions (Stokes and Robinson, 1949; Young, 1967; Rockland, 1960; Labuza et al., 1976; Greenspan, 1977). Unfortunately, the literature does not agree on the exact a_w of each salt solution and the effect of temperature. Thus, it is difficult to calibrate instruments and to evaluate a_w measuring devices, especially between laboratories (Chirife et al., 1983). The Greenspan (1977) review raised considerable doubt about the accuracy of the a_w of saturated salt solutions, although his values are commonly employed. Greenspan proposed the best a_w values for various salts at different temperatures; however, these values could also be criticized. The a_w values were computed as a function of temperature by using an empirical polynomial equation fitted to data published between 1912 and 1968 which were collected by a wide variety of methods, including direct measurement and gravimetric methods. Given the instrumental errors in the earlier studies, more recent measurements on advanced instruments with better accuracy are needed.

Based on the previous discussion, the present study was conducted to determine: (1) the a_w of saturated salt solutions as a function of temperature using an enhanced vapor pressure manometric method, (2) a method to obtain moisture sorption isotherms at high temperature so that better estimates of Q_s could be made, (3) the effect of a temperature shift on a_w changes in fish flour and cornmeal prepared to constant moisture content, and (4) the relationship of acutal a_w values for the shift study to the ones found from the sorption isotherm at the shift temperature as well as to those predicted by the Clausius Clapeyron relationship.

MATERIALS & METHODS

Materials

The fish flour used in this study was shipped in plastic packaging from Morocco in the summer of 1982. Upon arrival, the product was transferred to sealed glass bottles. The sealed bottles were then stored at 4°C for the duration of the study. Degerminated yellow corn flour was obtained in 100 pound bags from Krause Milling Company (Milwaukee, WI). The corn flour was also stored at 4°C in a sealed container.

Methods

Moisture content. The moisture content of fish flour and cornmeal used in this study was determined by the AOAC (1980) vacuum oven method using 10 hr at 70°C and 28″ Hg vacuum.

Water activity measurement. Eight reagent grade salts were used. The salts were dissolved in hot distilled water (100°C) and cooled to each test temperature (25, 30, 45°C) for crystallization to form a saturated slush. The vapor pressure manometer (VPM) used to measure the a_w of the salts was similar to the one described by Lewicki et al. (1978), except a transducer piezo-electric pressure gauge was used in place of the oil manometer as described by Troller (1983). The VPM was enclosed in a 0.25 in. (0.635 cm) plywood box lined with 2 inches (5.08 cm) of plastic foam to maintain temperature to within ± 0.1°C. Up to ten measurements of vapor pressure were made for each salt at each temperature.

Moisture adsorption isotherms. Moisture adsorption isotherms of fish flour and cornmeal were determined at 25, 30 and 45°C using the principles based on Labuza (1984). Two to three gram samples were placed in weighed sample dishes and dehydrated in a vacuum oven at 70°C for 8 hr. After drying, triplicate samples were placed in desiccators containing saturated salt slurries in the range 0.11-0.85 a_w. These were then held at either 25, 30 or 45°C. The slurries were checked at seven day intervals to ensure saturation. Equilibrium was judged to have been attained when the difference between two consecutive weekly sample weighings was less than 1 mg/g solids (generally 14-21 days). Since the desiccators could not be used above 45°C, the moisture adsorption isotherm at 65°C was determined by a flow method using the special chamber shown in Fig. 2. It consisted of three 1-L flasks containing saturated salt solutions of the same a_w. Between these flasks and the sorption chamber, a 1-L flask was used as an entrainment device to prevent condensation of excess water in the sorption chamber as air was

APPARATUS TO MEASURE SORPTION ISOTHERM AT HIGH TEMPERATURE

Fig. 2—Schematic of set up used to determine moisture sorption isotherms above 45°C.
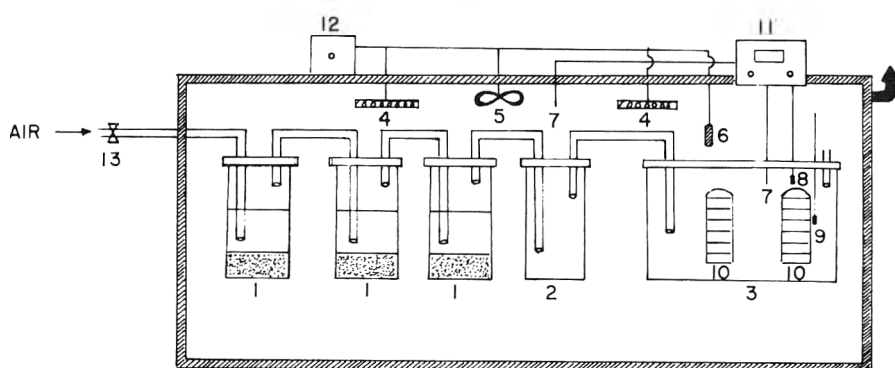
1 SALT SATURATERS	8 WET BULB THERMOCOUPLE
2 CONDENSOR	9 WET BULB THERMOMETER
3 SORPTION CHAMBER	10 SAMPLE HOLDER
4 HEATER	11 DIGITAL DISPLAY
5 FAN	12 TEMPERATURE CONTROLLER
6 TEMPERATURE SENSOR	13 AIR FLOW CONTROLLER
7 DRY BULB THERMOCOUPLE	

bubbled through. The four flasks were connected to the sorption chamber where the samples were placed. Wet and dry bulb thermo-couples were inserted into the sorption chamber in order to check the relative humidity. It was found that at 65°C, 10-12 hr were sufficient for the sample to reach equilibrium. The data for all the isotherms are shown in Table 1. The a_ws of the salts are the corrected ones which will be explained later.

Effect of temperature shift on a_w change at constant moisture content. To measure the effect of a temperature shift on the change in a_w at constant moisture content, a DT Kaymont Hygroscope (Kaymont Instruments, Huntington Station, NY) was used. The food sample was first equilibrated to different a_ws at 25°C. The DT Kaymont instrument was calibrated at each temperature by exposure to the same saturated salt solutions used for the desiccators. The food samples equilibrated at 25°C were taken from the desiccator and placed into the measuring chamber of the instrument which was connected to a circulating water bath with the temperature set to 25°C ± 0.1°C. When equilibrium was reached, as noted by no change in the readings over a 1-hr period, the instrument a_w value was read and the temperature was then shifted to 30°C. After again reaching equilibrium, the a_w was read again and the test was repeated at 45°C. The data collected was compensated according to the calibration curve for each specific temperature. The moisture loss from the sample into the head space of the Kaymont Hygro-scope evaluated from the gas law was found to be negligible and thus constant moisture was assumed, a necessary point for using Eq. (1). For example, at 45°C for an a_w of 0.8, the equilibrium moisture content of fish flour is 14g H_2O/100g solids. The sample weight used in the instrument was 5g which contains 614 mg of water. For a temperature shift of 25 going to 45°C, the moisture loss into the head space is about 0.1 mg. This is small compared to the total of 614 mg. From this it may be concluded that the instrument can be used to study the effect of temperature shift on a_w at constant moisture content. The temperature shift study was done in triplicate and the average a_w is reported which is the corrected value using the calibration curve at the temperature of the test.

RESULTS & DISCUSSION

Water activity of saturated salt solutions

The a_w values of salt solutions as measured by the VPM method at 25, 30 and 45°C are shown in Table 2 in comparison to the Greenspon (1977) values. It should be noted that the standard deviation for all measurements was very small and that there are significant differences from the

Greenspan data (at the 95% level). The low standard deviation indicates that the VPM method has good repeatability. As seen, the a_w of the salt solutions decreases with an increase in temperature. This is in agreement with the results obtained by Troller (1983) using the capacitance VPM, and close to those of Chikao et al. (1976) but are opposite to Scott and Bernard (1981).

Regression analysis using the least square method on ln a_w versus $1/T$ °K for each salt is shown in Table 3 (Young, 1967). For all equations, the r^2 is between 0.96 and 0.99. Table 3 also shows that the temperature effect on the a_w of salt solutions is not the same for all salts. This is because salt solutions have different heats of solution, as can be seen from the slope of the different equations. The slope is related to the "total apparent heat of water sorption" (Q_{as}) for saturated salt solutions. Q_{as} is used to avoid confusion with the heat of sorption of water in the case of food, since these heats do not measure the same quantity of energy. The difference is due to the fact that the Clausius Clapeyron equation applies only when the moisture content is held constant. This is not the case for the salt solutions since at higher temperature more salt is needed to reach saturation and thus the moisture content decreases. The heat absorbed (ΔH_T) in this process includes both the enthalpy of vaporization of water from the given salt solution (ΔH_1) and the enthalpy of solvation of the solute into the solution (ΔH_2). Hence:

$$\Delta H_T = \Delta H_1 + \Delta H_2 \qquad (2)$$

$$\Delta H_T = \Delta H_1 + Q_s \qquad (3)$$

where: ΔH_T = the heat of vaporization of water from the saturated salt solution + the heat of solution of the solute into the solution; ΔH_1 = the heat of vaporization of water from the solution; ΔH_2 = the heat of solution of the solute in H_2O; ΔH_L = latent heat of vaporization of pure water; Q_s = the extra heat needed to evaporate water from the salt solution due to the interaction of water with the solute.

It follows that the slope of ln a_w versus $1/T$ will be equal to the heat of solution of the solute in water (ΔH_2) plus the extra heat needed to evaporate water from the saturated salt solution (Q_s), i.e., $Q_{as} = Q_s + \Delta H_2$. Thus, the

Table 1—Water Adsorption Isotherms of Fish Flour and Cornmeal as a Function of Temperature

Fish flour Salts	25°C		30°C		45°C		65°C	
	a_w	g H_2O/100 g solids	a_w	g H_2O/100 g solids	a_w	g H_2O/100 g solids	a_w	g H_2O/100 g solids
LiCl	0.115	2.12	0.110	1.69	0.103	1.52	0.20	1.85
$KC_2H_3O_2$	0.234	3.83	0.231	3.32	0.197	2.73	0.27	2.65
$MgCl_2$	0.329	5.53	0.325	4.50	0.307	3.75	0.35	3.52
K_2CO_3	0.443	6.82	0.437	5.62	0.429	4.60	0.43	4.38
$Mg(NO_3)_2$	0.536	7.65	0.521	6.44	0.496	5.40	0.51	5.11
$NaNO_2$	0.654	10.29	0.648	8.42	0.599	7.30	0.64	7.85
NaCl	0.765	13.40	0.748	13.20	0.727	11.83	0.70	9.63
KCl	0.848	17.50	0.841	16.70	0.786	14.30	0.73	10.59
							0.80	13.93

Cornmeal Salts	a_w	m	a_w	m	a_w	m	a_w	m
LiCl	0.115	4.69 ± 0.01	0.110	4.46 ± 0.15	0.103	3.39 ± 0.13	0.20	4.17 ± 0.01
$KC_2H_3O_2$	0.234	7.44 ± 0.12	0.231	6.35 ± 0.11	0.197	5.34 ± 0.07	0.37	6.33 ± 0.06
$MgCl_2$	0.329	9.12 ± 0.04	0.325	8.12 ± 0.20	0.309	7.07 ± 0.07	0.45	7.56 ± 0.03
K_2CO_3	0.443	11.04 ± 0.05	0.437	9.90 ± 0.16	0.429	8.39 ± 0.01	0.54	8.90 ± 0.17
$Mg(NO_3)_2$	0.536	11.91 ± 0.01	0.521	10.43 ± 0.34	0.496	8.75 ± 0.02	0.58	9.23 ± 0.23
$NaNO_2$	0.654	13.14 ± 0.14	0.648	12.90 ± 0.28	0.599	10.80 ± 0.08	0.63	9.30 ± 0.16
NaCl	0.765	15.47 ± 0.33	0.727	13.69 ± 0.09	0.727	13.69 ± 0.09	0.70	12.78 ± 0.45
KCl	0.846	19.58 ± 0.00	0.841	19.51 ± 0.39	0.786	17.04 ± 0.64	0.72	13.26 ± 0.58
							0.80	15.04 ± 0.16

Table 2—Values of water activity of saturated salt solutions at different temperatures as compared to the Greenspan (1977) values

Salts	25°C	Greenspan	30°C	Greenspan	45°C	Greenspan
LiCl	0.115[a] (0.003)[b]	0.113	0.110 (0.003)	0.113	0.103 (0.004)	0.112
$KC_2H_3O_2$	0.234 (0.003)	0.225	0.236 (0.002)	0.22	0.197 (0.002)	- - -
$MgCl_2$	0.329 (0.003)	0.327	0.326 (0.004)	0.32	0.309 (0.002)	0.311
K_2CO_3	0.443 (0.001)	0.432	0.437 (0.001)	0.431	0.429 (0.002)	- - -
$Mg(NO_3)_2$	0.536 (0.003)	0.530	0.521 (0.003)	0.541	0.496 (0.001)	0.470
$NaNO_2$	0.654 (0.002)	- - -	0.648 (0.003)	- - -	0.599 (0.002)	- - -
NaCl	0.765 (0.003)	0.750	0.748 (0.003)	0.751	0.727 (0.004)	0.745
KCl	0.846 (0.002)	- - -	0.841 (0.002)	0.836	0.786 (0.001)	- - -

[a] Water activity
[b] Standard deviation

Table 3—$\ln a_w$ versus $1/T$ regression equations

Salts	Regression equation	r^2
LiCl	$\ln a_w = 500.95\ 1/T - 3.85$	0.976
$KC_2H_3O_2$	$\ln a_w = 861.39\ 1/T - 4.33$	0.965
$MgCl_2$	$\ln a_w = 303.35\ 1/T - 2.13$	0.995
K_2CO_3	$\ln a_w = 145.0\ \ 1/T - 1.3$	0.967
$MgNO_3$	$\ln a_w = 356.6\ \ 1/T - 1.82$	0.987
$NaNO_2$	$\ln a_w = 435.96\ 1/T - 1.88$	0.974
NaCl	$\ln a = 228.92\ 1/T - 1.04$	0.961
KCl	$\ln a = 367.58\ 1/T - 1.39$	0.967

Clausius Clapeyron will have the following form if one assumes constant composition, which over a small temperature range for salts is not a bad assumption.

$$\ln \frac{a_2}{a_1} = \frac{-(Q_s + \Delta H_2)}{R}\left[\frac{1}{T_2} - \frac{1}{T_1}\right] \tag{4}$$

The slope of a plot of $\ln a_w$ versus $1/T$ should give:

$$\text{slope} = -\left[\frac{Q_s + \Delta H_2}{R}\right] \tag{5}$$

As seen in Table 3, the slope for all salts has a positive value which means that $Q_s + \Delta H_2$ is negative. Since Q_s should be positive, it follows that ΔH_2 is negative and its absolute value is higher than Q_s. This is true for most of the salt solutions. The heats of solution at infinite dilution for some salts are available. For example, for LiCl, $Mg(NO_3)_2$, and KCl, they are respectively -8850, -3700, and -4404 cal/mole (Perry, 1973). The ΔH_2 values for saturated solutions are not available. Assuming that one can use the values at infinite dilution, the negative value of H_2 would cause the slope to be positive, resulting in a decrease in a_w with increased temperature.

Thermodynamically, the chemical potential of component A in an ideal solution, μ_{A_L}, is:

$$\mu_{A_L} = \mu_A^\circ + RT \ln a_{A_L} \tag{6}$$

where μ_A° = the chemical potential of pure liquid A and a_A is the activity of species A. Since at equilibrium in solid state, the activity of A is one, then:

$$\mu_{A_S}^\circ = \mu_A \tag{7}$$

Thus, for one mole:

$$\frac{G_{A_L} - G_{A_S}}{RT} = \ln a_{A_L} \tag{8}$$

Since we know that a_A is equal to the mole fraction of species $A(X_A)$, then differentiating with respect to temperature we get:

$$\frac{\partial(\ln X_A)}{\partial T} = -\frac{(H_{A_L} - H_{A_S})}{RT^2} = -\frac{\Delta H_T}{RT^2} \tag{9}$$

where: H_{A_L} = the partial molar enthalpy of the component A in the ideal solution; H_{A_S} = the partial molar enthalpy of pure solid A; ΔH_T = the difference in enthalpy which should be a positive value since $H_{A_S} > H_{A_L}$.

If X_w = mole fraction of water in the saturated salt solution, then:

$$X_A = 1 - X_w \tag{10}$$

and, Eq. (9) at constant pressure, p, becomes:

$$\left[\frac{\partial[\ln(1 - X_w)]}{\partial T}\right]_p = -\frac{\Delta H_T}{RT^2} \tag{11}$$

Also, we know that a_w = the water activity = $\gamma_w X_w$ where γ_w = the activity coefficient for water. Thus, with respect to $1/T$, Eq. (11) becomes:

$$\left[\frac{\partial \ln(1 - \frac{a_w}{\gamma_w})}{\partial(\frac{1}{T})}\right]_p = -\frac{\Delta H_T}{R} \tag{12}$$

Thus, a plot of $\ln\left[1 - \frac{a_w}{\gamma_w}\right]$ versus $1/T$ should be a straight line if ΔH_T does not vary with temperature. Regression of this equation was not possible since that value of γ_w is not known. Assuming a value of 1 for γ_w gave r^2 of 0.99 or greater but it should be obvious that γ_w is not 1.

Eq. (12) thus shows that for saturated salt solutions, the a_w should decrease with increasing temperature, as has been found in the present results and reported in most of the literature where direct vapor pressure was measured. This

suggests that the opposite effect found by Scott and Bernard (1983) was due to errors in calibration of the hygrometer. Table 2 also shows the a_w values measured on the VMP as compared to the values from empirical polynomials of Greenspan (1977). A t test showed that all VPM values differed from those of Greenspan (1977) at the 95% confidence level. These VPM values were used in the rest of the study.

Water sorption isotherms of fish flour and cornmeal

The adsorption isotherms of fish flour and cornmeal determined at 25, 30, 45 and 65°C are presented respectively in Table 1 and in Fig. 3 and 4 using the corrected a_ws for the salts at each temperature. All of the isotherms gave the characteristic S shaped curve of normal moisture adsorption isotherms (Adamson, 1979). It is clearly evident from the figures that for any constant moisture content, an increase in temperature significantly increases the a_w. A review of at least 150 published papers with isotherm data shows that many workers present sorption isotherms at high temperatures by plotting equilibrium moisture content versus the a_w values of salt solutions as determined at 25°C, rather than versus the true a_w of the salt solution at the temperature of the study. The error can be clearly seen in Fig. 5, where both the uncorrected and corrected isotherms are plotted at 45°C versus the true isotherm at 25°C. As noted, not correcting isotherm a_w values distorts its shape and makes the magnitude of the shift seem larger than it actually is.

Application of BET and GAB equations

In an extensive review of sorption models proposed in the literature, Van Den Berg and Bruin (1981) stated that the "GAB" equation (from Guggenheim-Anderson-de Boer) was the best theoretical model for foods. It is a three parameter equation and is the accepted model used by all European food researchers (Bizot, 1983). The equation is:

$$\frac{m}{m_o} = \frac{Cka_w}{(1 - ka_w)(1 - ka_w + Cka_w)} \qquad (14)$$

where: a_w = water activity; m = equilibrium moisture content on dry basis; m_o = monolayer moisture content; C C = Guggenheim constant = $C'exp(H_1 - H_m)/RT$; H_1 = heat of condensation of pure water vapor; H_m = total heat of sorption of the first layer; k = factor correcting proper-

ties of multilayer molecules with respect to the bulk liquid = $k'exp(H_1 - H_q)/RT$; H_q = total heat of sorption of the multilayer.

An HP9816 computer was used on the isotherm data of Table 1 to perform the nonlinear regression needed to determine the equation constants. It should be noted that all the data can be used, while in the BET model data above $a_w = 0.45$ is precluded. The curves drawn in Fig. 3 and 4 are based on the computer solution. The standardized residual errors showed no systematic departures as a function of a_w indicating excellent fit.

The values of the monolayer moisture content at each temperature as calculated by the GAB and BET equation are presented in Table 4. The results show that the monolayer moisture content decreases with increasing temperature between 25-65°C, except for fish flour at 65°C. This behavior has also been reported for almost 100 different foods and food systems by Iglesias and Chirife (1976a, b). A t test showed that the monolayer moisture contents as obtained by the GAB equation do not differ from those obtained by the BET equation at the 95% level of significance for each temperature. The GAB equation has an

MOISTURE SORPTION ISOTHERMS CORN MEAL
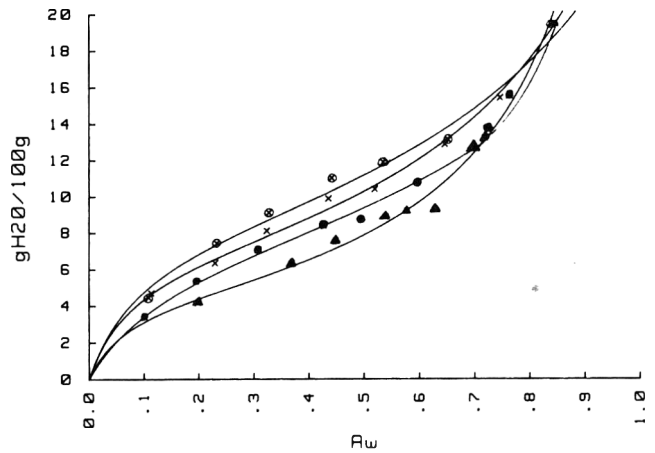
Fig. 4—Moisture sorption isotherms for cornmeal: ⊗ 25°C, x 35°C, ● 45°C, ▲ 65°C.

MOISTURE SORPTION ISOTHERMS (FISH FLOUR)

Fig. 3—Moisture sorption isotherms for fish flour: ⊗ 25°C, x 35°C, ● 45°C, ▲ 65°C.

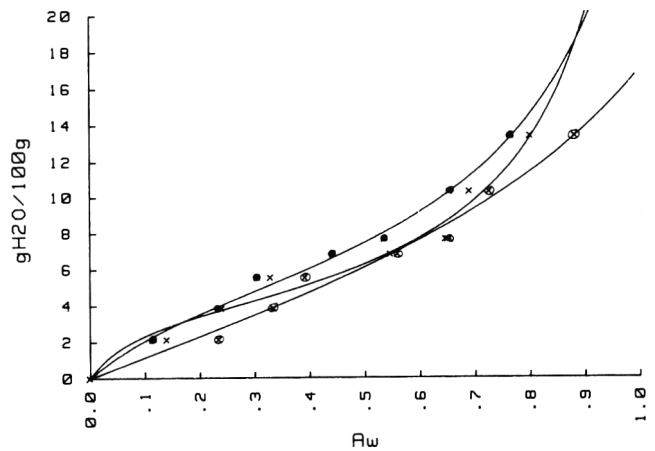

Fig. 5—Comparison between corrected (true salt a_w at 45°C x) and uncorrected (a_w value of salt from 25°C ⊗) isotherms at 45°C versus the true isotherm (●) at 25°C for fish flour.

Table 4—Monolayer Moisture Content (m_O) g H_2O/100 g solids

	GAB	BET
Fish flour		
25°C	5.12	4.33
30	4.33	3.65
45	3.24	3.15
65	3.94	3.30
Cornmeal		
25°C	8.23	6.86
30	6.50	6.10
45	5.68	4.89
65	5.57	4.66

Table 5—Excess heat of sorption (Q_S) for cornmeal and fish flour as a function of moisture content

Moisture g H_2O/100g solids	Kcal/mole	
	Corn meal	Fish flour
2	4.45	2.80
4	4.30	2.59
6	3.91	1.50
8	2.74	0.60
10	1.76	0.40
12	1.11	0.25
14	0.71	0.20
16	0.43	—
20	0.20	—

Table 6—Comparison of water activity shift as measured on the Kaymont instrument (corrected with actual isotherm values) and the Clausius Clapeyron equation prediction

g H_2O/100g solids	Q_S* Kcal/mole	25°C Isotherm	25°C Measured	35°C Measured	35°C Isotherm	35°C Equation**	45°C Measured	45°C Isotherm	45°C Equation**
Fish flour									
2.12	2.8	0.115	0.111	0.115	0.14	0.129	0.140	0.15	0.149
3.83	2.6	0.234	0.221	0.223	0.27	0.255	0.240	0.31	0.29
5.53	1.6	0.329	0.328	0.327	0.33	0.358	0.385	0.50	0.388
7.65	0.7	0.536	0.540	0.545	0.60	0.561	0.651	0.62	0.582
10.29	0.43	0.654	0.643	0.637	0.70	0.658	—	0.71	0.673
13.40	0.21	0.765	0.754	0.725	0.78	0.762	0.800	0.78	0.771
Cornmeal									
4.69	4.2	0.115	0.078	0.093	0.11	0.098	0.112	0.16	0.12
7.44	3.0	0.234	0.205	0.226	0.27	0.24	0.271	0.35	0.28
9.12	2.2	0.329	0.314	0.338	0.40	0.354	0.405	0.50	0.40
11.04	1.47	0.443	0.433	0.462	0.54	0.469	0.534	0.61	0.51
11.91	1.10	0.536	0.525	0.522	0.59	0.56	0.609	0.64	0.59
13.14	0.91	0.654	0.601	0.620	0.65	0.63	0.697	0.69	0.66
15.59	0.48	0.765	0.686	0.721	0.74	0.71	0.810	0.76	0.72
19.58	0.2	0.846	0.799	0.833	0.84	0.81	0.875	0.83	0.816

* Estimated from Q_S versus moisture data of Table 5
** Calculated from Clausius Clapeyron equation using the 25°C measured value for the prediction

advantage as compared to the BET model since it offers an objective method for drawing sorption isotherms up to 0.9 a_w, while the BET model is limited to 0.45-0.5 a_w. Table 5 presents the Q_S values as a function of moisture content using linear regression of the a_ws at each of the four temperatures (Eq. 1). Cornmeal has a higher sorption heat probably because it is predominantly carbohydrate and has more polar groups. Above 14g H_2O/100g solids, the excess heats are small for both systems (<500 cal/mole) as compared to the heat of vaporization (10.5 Kcal/mole) which is typical of most foods (Iglesias and Chirife, 1976b).

Temperature shift study

The effect of a temperature shift on change in a_w at constant moisture content required the calibration of the Kaymont instrument at each temperature for the shift experiment. The calibration curves used all salts except K_2CO_3, as it interacted with the cell at each temperature. This calibration was based on the AOAC (1980) standard procedure. Straight lines with an r^2 of >0.98 were obtained. Fig. 6 shows the resulting a_w shift for cornmeal at constant moisture content due to the temperature shift. It is evident from Fig. 6 that for any moisture content, an increase in temperature induces an increase in a_w. For example, at 7% dry basis moisture content, the a_w change is about 0.1 for a temperature shift from 25 to 45°C.

Table 6 shows the measured a_w found for the 30° and

45°C temperature shift experiment using the Kaymont a_ws, the a_w at the test moisture content at 30° and 45°C interpolated from the GAB isotherm equation at each temperature, and the a_w predicted using the Clausius Clapeyron equation and the Q_S values of Table 5 at the test moisture content. For fish flour at 35°C, the a_w shift found using the Kaymont was about 0.01-0.02 units less than that predicted from the Clausius Clapeyron equation for a_ws below 0.65 and from 0.01-0.05 units less than found from what should be the true isotherm, i.e., that using the corrected VPM a_w values. At 45°C, however, the measured a_w in the shift test was higher than the GAB value or Clausius Clapeyron value above 0.65 a_w. For the cornmeal, even at 25°C, the measured a_ws of the starting material were always less than the true isotherm a_w values. This could be due to the use of the regression lines or possibly nonequilibrium, although storage was at 25°C for 2 wk. Thus, the measured values at 35°C and 45°C are less than the true isotherm, except for 45°C above an a_w of 0.65. The measured a_ws were generally within 0.01 units of the predicted value from the Clausius Clapeyron equation at 35°C, and at 45°C below 0.65 a_w.

Overall, this study shows that the Clausius Clapeyron equation predicts the shift in a_w with temperature quite well while the isotherm at the actual shift temperature shows a higher value than the a_w measured by a hygrometer. Since the difference is less than 0.02 a_w units in most
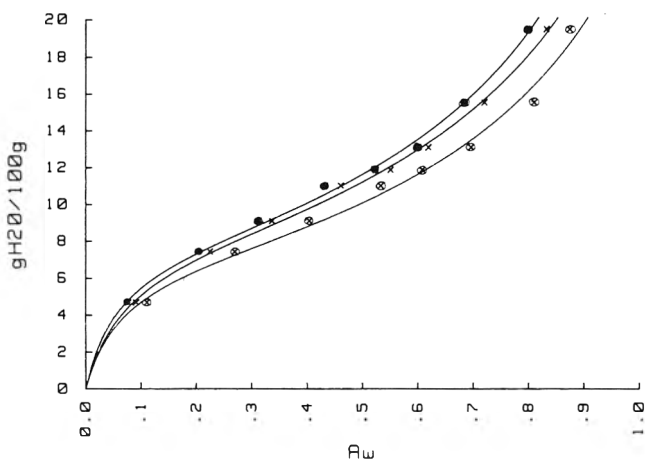
Fig. 6—Water activity shift for cornmeal when shifted to 35°C and 45°C from 25°C for constant moisture contents: ● 25°C, x 35°C, ⊗ 45°C.

cases, using the hygrometer in a temperature shift experiment should give an adequate estimate of the a_w at the higher temperature without having to make the actual isotherm. It is also possible that, at the higher temperature and a_ws where larger differences occurred (higher a_w from instrument), the 4-6 wk storage in creating the actual isotherm at 45°C decreased the sorption capacity of the food material due to chemical reactions. In the shift test, the sample at most was exposed to 45°C for less than 24 hr. It is also possible that in this time the water did not re-equilibrate and may have been more lossely bound. Overall, it can be stated that the instrument method used gives an adequate estimate of the actual shifts that could occur.

REFERENCES

AOAC. 1980. Water activity: Canned Products Method 32.004-32.009. Association of Official Analytical Chemists, Washington, DC.

Adamson, A.W. 1979. "A Textbook of Physical Chemistry," 2nd ed. Academic Press, Inc., New York, NY.

Bizot, H. 1983. Using "G.A.B." model to construct sorption isotherms. In "Physical Properties of Food." (Ed.) Jowitt et al. Applied Science Publishers, London and New York.

Bruin, S. and Luyben, K.C. 1980. Drying of food materials: a review of recent developments. In "Advances in Drying," Vol. 1, p. 155. Hemisphere Publishing Company, Washington, New York and London.

Brunauer, S., Emmett, P.H., and Teller, E. 1938. Adsorption of gases in multimolecular layers. J. Am. Chem. Soc. 60: 309.

Carr, D.S. and Harris, B.L. 1949. Solutions for maintaining constant relative humidity. Ind. Eng. Chem. 41: 2014.

Chikao, A., Hosaka, S., Murase, K., and Saus, Y. 1976. Measurement of the relative humidity of saturated salt solutions. J. Chem. Eng. (Japan) 9(4): 673.

Chirife, J., Guillemo, F., Constantino, F.F., and Silvia, L.R. 1983. The water activity of standard saturated salt solutions in the range of intermediate moisture foods. Lebensm. Wiss. Technol. 16: 1.

Gal, S. 1981. Recent developments in techniques for obtaining complete sorption isotherms. In "Water Activity: Influences on Food Quality," (Ed.) L.B. Rockland and G.F. Stewart. Academic Press, Inc., New York, NY.

Graciala, N.R., Urbicain, M.J., and Rotstein, E. 1982. Moisture equilibrium in apples at several temperatures: experimental data and theoretical considerations. J. Food Sci. 47: 1448.

Greenspan, L. 1977. Humidity fixed points of binary saturated aqueous solutions. J. Res. Nat'l. Bureau of Standards-Section A. Physics & Chemistry. 81A(1).

Iglesias, H.A. and Chirife, J. 1976a. Prediction of the effect of temperature on water sorption isotherms of food material. J. Food Technol. 11: 109.

Iglesias, H.A. and Chirife, J. 1976b. Isosteric heats of water sorption on dehydrated foods, Part 1. Analysis of the differential heat curves. Lebensm. Wiss. Technol. 9(2): 116.

Iglesias, H.A. and Chirife, J. 1982. "Handbook of Food Isotherms." Academic Press, New York.

Labuza, T.P. 1968. Sorption phenomena in food. Food Technol. 22(3): 15.

Labuza, T.P. 1970. Properties of water as related to the keeping quality of foods. Proceedings, 3rd Int'l. Congress, Food Science and Technology. IFT, p. 618.

Labuza, T.P. 1984. "Moisture Sorption: Practical Aspects of Isotherm Measurement and Use." American Association of Cereal Chemists, St. Paul, MN.

Labuza, T.P., Acott, K.A., Tatini, S.R., Lee, R., Flink, J., and MacCall, W. 1976. Water activity determination — A collaborative study. J. Food Sci. 41: 910.

Lewicki, P.P., Busk, G.C., Peterson, P.L., and Labuza, T.P. 1978. Determination of factors controlling accurate measurement of a_w by the vapor pressure manometric technique. J. Food Sci. 43: 244.

Loncin, M. 1980. Water adsorption isotherms of foods at high temperature. Lebensm. Wiss. Technol. 13: 182.

Loncin, M., Lenges, J., Bimbenet, J.J., and Jacqman, D. 1965. L humidity relative d equilibre, son importance et sa mesure. Ind. Alim. Agric. p. 923.

Perry, J.H. 1973. "Chemical Engineering Series," 5th ed. McGraw Hill. Rockland, L.B. 1960. Saturated salt solutions for static control of relative humidity between 5 and 110°C. Anal. Chem. 32: 10.

Salwin, H. 1959. Defining minimum moisture contents for dehydrated foods. Food Technol. 13: 594.

Scott, W.J. 1957. Water relations of food spoilage microorganisms. Adv. Food Res. 7: 83.

Scott, V.N. and Bernard, D.T. 1983. Influence of temperatures on the measurement of water activity of food and salt systems. J. Food Sci. 48: 552.

Stokes, R.M. and Robinson, R.A. 1949. Vapor pressures for inorganic salts. Ind. Eng. Chem. 41: 2013.

Troller, J.A. 1983. Water activity measurements with a capacitance manometer. J. Food Sci. 45: 739.

Troller, J.A. and Christian, J.H.B. 1978. "Water Activity and Foods." Academic Press, Inc., New York, San Francisco and London.

Van Den Berg, C. and Bruin, S. 1981. Water activity and its estimation in food systems: theoretical aspects. In "Water Activity: Influence on Food Quality." (Ed.) L.B. Rockland and G.F. Stewart, p. 1. Academic Press, Inc., New York, NY.

Young, J.F. 1967. Humidity control in the laboratory using salt solutions — a review. J. Appl. Chem. 17: 241.

Finite Element Analysis of Moisture Diffusion in Stored Foods

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ABSTRACT

A Finite Element model was used to predict the average moisture content of foods represented by axisymmetric shapes. The model was found to adequately predict the moisture content for the majority of the foods represented by cereal, dairy, fruit, vegetable, meat and semi-moist bakery food groups. For two foods, flour desorbing to 0.11 a_w and turnips adsorbing to 0.75 a_w, the predictions improved when variable diffusivity was used as contrasted with constant diffusivity. The Finite Element model was tested for different foods with different size, shape and diffusion potential.

INTRODUCTION

A MATHEMATICAL MODEL can be used as a tool to predict the moisture content of foods during storage over time at a given water activity (a_w). The moisture distributions with time could also be predicted, as well as ingredient mixing. With modifications, the model could be used to describe the shelf life of packaged foods. Mathematical modeling can also be used for the prediction of drying times and of packaging and storage requirements.

Exact solutions to differential equations governing moisture movement can be obtained for fixed geometries: sphere, slab or a cylinder (Crank, 1975). The solutions are obtained by assuming constant material properties (i.e., diffusion coefficient), but when the properties become dependent upon location or concentration the solutions become more complicated and often do not exist (Polivka and Wilson, 1976). Numerical techniques can be utilized for the more complex but truer to real life situations. Among numerical methods available to solve the governing differential equations for moisture transfer are the Finite Difference and the Finite Element analyses. The Finite Difference method of analysis is based on the approximation by difference of a derivative at a point (Naveh et al., 1983). Solutions by the Finite Difference method often result in long computational times, and often the material properties are difficult to vary from node to node (Misra and Young, 1979). In addition, the analysis is often limited to cases where the body has (or can be approximated by) a simple geometry (Naveh et al., 1983). The Finite Element method of analysis is a powerful method of analysis which overcomes the above mentioned problems. Zhang et al. (1984) used the Finite Element method to model water diffusion in rice with diffusion coefficient as a function of moisture concentration, and they also were able to account for the change in the size of rice during soaking. The fundamental concept of the Finite Element method of analysis is that any continuous quantity, such as moisture content or temperature, can be approximated by a discrete model composed of a set of piecewise continuous quantity, such as moisture content or temperature, can be approximated by a discrete model composed of a set of piecewise continuous functions defined over a finite number of domains (Segerlind, 1976).

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The objectives of this study were: (1) To discuss the development of a Finite Element model to describe moisture diffusion in stored food products represented by axisymmetric shape with constant and variable moisture transfer properties, and (2) To compare the model predicted moisture contents to the experimental values.

MATERIALS & METHODS

ALL OF THE FOODS used in this study were purchased from a local supermarket. The foods were selected in an attempt to represent different food groups. The foods and representative food groups included: flour (cereal), nonfat dried milk (dairy), freeze-dried apple (fruit), freeze-dried turnip (vegetable), freeze-dried raw ground beef (meat), oatmeal cookies (semi-moist bakery product), shredded wheat (cereal), and raisins (slow sorbing, dense product). The sorption experiments were carried out in desiccators and Mason jars at 25 ± 1°C. All of the foods were adsorbed to 0.75 a_w. The flour was also adsorbed and desorbed to 0.52 and 0.11 a_w, respectively, with the intent of testing the model for a different driving force.

Saturated salt solutions were used to generate a constant equilibrium relative humidity within the desiccators, Mason jars or fish tanks. The details of the experiment and equilibrium moisture contents (Table 1) for the foods are as given by Lomauro et al. (1984). In general, the food samples were 3 – 10 mm in thickness and 50 mm in diameter. In addition the flour was adsorbed to 0.75 a_w for three thickness and one pyramid shape to test the Finite Element model.

The initial moisture contents of the food samples were determined by the Karl Fischer method (Fischer, 1935) using an Aquatest IV titrator (Photovolt Corporation, New York, NY). The apparatus measures moisture content by relating change in current to a change in free iodine content.

The effective diffusion coefficient (Deff) was calculated for each of the food products. The details were as given by Lomauro et al. (1985).

RESULTS & DISCUSSION

Governing equation and boundary conditions

The differential equation for transient moisture diffusion in an anisotropic, nonhomogeneous material representing an axisymmetric body in cylindrical coordinates is:

$$\frac{\partial}{\partial r}\left(rD_r \frac{\partial M}{\partial r}\right) + D_r \left(\frac{\partial M}{\partial r}\right) + \frac{\partial}{\partial z}\left(rD_z \frac{\partial M}{\partial z}\right) = r\frac{\partial M}{\partial t} \quad (1)$$

where r and z are the coordinate directions, D_r and D_z are the mass diffusivities in the coordinate directions, M is the moisture content and t is the time. Eq. (1) assumes no changes in dimension, swelling or shrinkage during the sorption.

The initial condition describing the body is defined by a function of r and z prescribing a moisture distribution throughout the body:

$$M(r,z,0) = f(r,z) \quad \text{at } t = 0 \quad (2)$$

Two types of boundary conditions can be given for Eq. (1). A prescribed moisture content on a surface S1:

$$M(r,z,t) = M_s; \quad r,z \text{ on a surface}; \quad t > 0 \quad (3)$$

and/or a convection type boundary condition on a surface S2:

$$D \frac{\partial m}{\partial N}(r,z,t) = h'(M' - M_s); \quad r,z \text{ on a surface}; \quad t > 0 \quad (4)$$

where D is the diffusion coefficient, h' is the surface mass transfer coefficient, M' is the moisture content of surrounding atmosphere, and M_s is the moisture content at the surface of the body. N is the outward normal to the surface.

The material properties D_r and D_z can be moisture concentration dependent. Boundary condition 1 (Eq. 3) can be a function of the time. The equations are defined for an axisymmetric body; therefore, Eq. 1 to 4 are assumed to be independent of the angle of rotation around the z axis.

Finite Element solution

The moisture distribution within an axisymmetric solid idealized by a system of Finite Elements may be derived in a number of ways (Polivka and Wilson, 1976). Naveh (1983) derived the system using variational calculus, where a functional form is found and any function that minimizes the integral of the function also satisfies the governing equation. Another method is the method of weighted residuals, which is based upon the minimization of a residual existing between the exact and approximate solution (Norrie and DeVries, 1978). Polivka and Wilson (1976) took a purely physical interpretation for the equations which govern heat flow equilibrium. Identical final matrix equations are obtained regardless of the method used (Naveh, 1983). The resulting matrix differential equation governing moisture diffusion in a Finite Element system containing n nodes is:

$$[C] \frac{d}{dt}[M] + [K][M] - F = 0 \quad (5)$$

$$\text{nxn} \quad \text{lxn} \quad \text{nxn lxn} \quad \text{lxn}$$

where [C] and [K] are square coefficient matrices, F is a column vector of known values and [M] is the unknown moisture content. The form of coefficient matrices is as given by Segerlind (1976). These equations were programmed on CDC computer.

A flow chart of the computer program is given in Fig. 1. Since it is known that the degree of accuracy of the Finite Element model is dependent upon the element size and time step increment, a small study was conducted to find an adequate time step increment and grid size for a representative food shape under study. By comparing the moisture content values as predicted by Finite Element model and analytical solution for a cylindrical shaped object, it was found that a grid containing at least 30 4-node isoparametric elements and a 1 hr time step increment was adequate for describing moisture diffusion.

Comparison of Finite Element prediction to analytical solution

To test the Finite Element program, a cylinderical shaped food product was modeled and checked against analytical solution for moisture content. The Finite Element calculations were done using two grid sizes (20 and 30 − 4 node isoparametric elements, and two time step increments (1 and 10 hr). The following conditions were used for the calculations for a food product represented by a finite cylinder: Diffusion coefficient = 0.33 m²/hr; Height = 10 mm, Diameter = 5.2 mm; Initial moisture content, Mo = 12.0g water/100g solid; Equilibrium moisture content, Me = 17.0g water/100g solid.

It was assumed that all surfaces instantaneously equilibrated with the atmosphere. The calculations for the analytical solutions for the center moisture were made using the first 60 terms of the following analytical solutions given by Crank (1975):

Infinite slab solution

$$\Gamma_{is} = \frac{M-Me}{Mo-Me} = \frac{4}{\pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{(2n+1)} \exp\left[\frac{-D(2n+1)^2\pi^2 t}{4a^2}\right]$$

where a = thickness of the slab.

Infinite cylinder solution

$$\Gamma_{ic} = \frac{M-Me}{Mo-Me} = \frac{2}{a} \sum_{n=1}^{\infty} \frac{\exp(-Db_n^2 t)}{b_n J_1(b_n a)}$$

where a = radius of the cylinder; b_n = roots of $J_0(b_n a)$. Thus, the center moisture content for the finite cylinder is:

$$M_{center} = [(\Gamma_{is} \times \Gamma_{ic})(Mo-Me)] + Me$$

The Finite Element predictions are compared with the analytical solution in Fig. 2. It can be seen that if a sufficient number of elements and small time increments are used, the Finite Element predictions are close to the analytical solution. Similar results were achieved for other geometries and for average moisture content (Lomauro et al., 1985). The analytical solutions are available for fixed geometric shapes only and that too for constant diffusivity. This may not be the case with most of the food products. For these products Finite Element models can be used. The Finite Element predictions for food products described
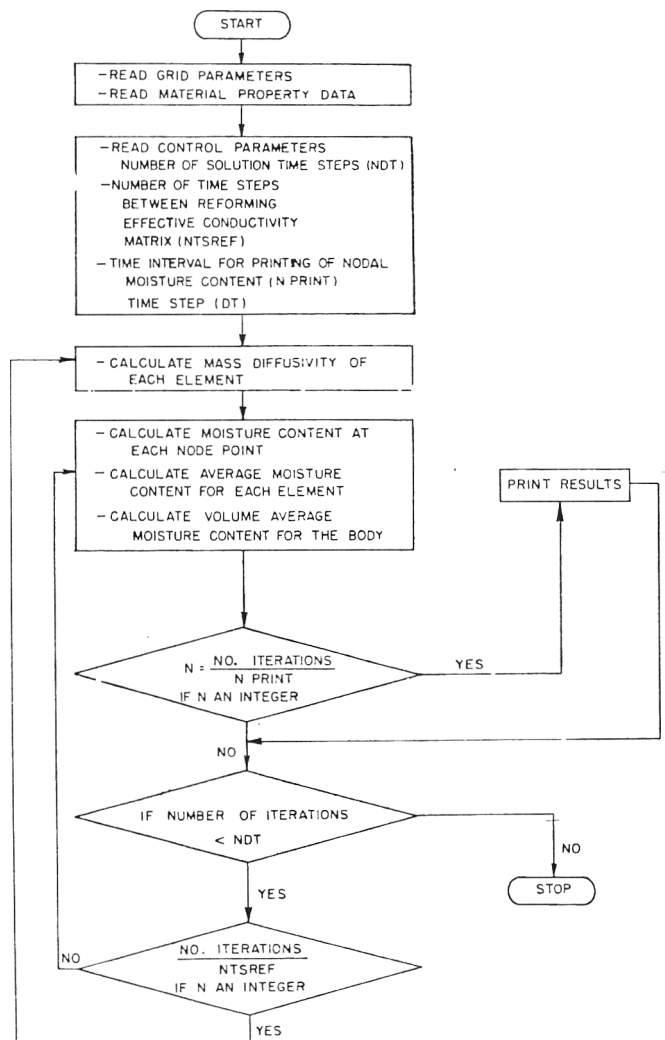

Fig. 1—Flow chart for the Finite Element program.

by both constant and variable diffusivities and represented by different shapes and sizes are discussed in the next section.

Products represented by a constant diffusivity

The Finite Element description for the foods listed in
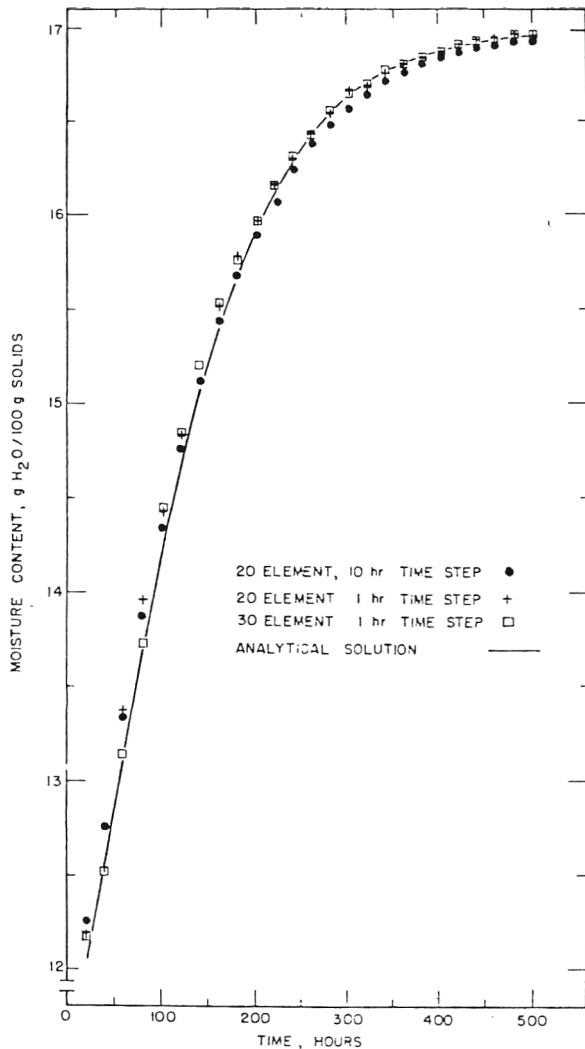
Fig. 2—Comparison of Finite Element predictions to an analytical solution for the center moisture content of a cylinderical shaped food (Height = 10 mm, Diameter = 5.2 mm, Deff = 0.33 mm²/hr)

Table 1—Parameters for food products in Finite Element model

Product	Adsorbing (A) Desorbing (D)	Diffusion coefficient m²/hr x 10⁷	Equilibrium moisture content g water/100g solids
Flour	D	0.139ᵃ	6.66
Flour	A	1.152	16.68
Nonfat dry milk	A	0.767	12.49
Freeze-dried apple	A	0.146	31.77
Freeze-dried turnip	A	0.274ᵇ	30.75
Freeze-dried raw ground beef	A	1.105	15.66
Oatmeal cookie	A	0.143	17.76
Shredded Wheat	A	0.199	10.68
Raisin	A	0.015	34.80

ᵃ (Deff)1 = 0.146 for $\Gamma = M - M_s/M_0 - M_s \geq 0.12$; (Deff)2 = 0.022 for $\Gamma = < 0.12$.
ᵇ (Deff)1 = 0.268 for $\Gamma = \geq 0.05$; (Deff)2 = 0.050 for $\Gamma = < 0.05$.

Table 1 is shown in Fig. 3. Each of the foods except the raisin is represented as a solid of rotation around the height axis (Fig. 3). The food was divided into 35 4-node isoparametric elements. The bottom of the food (hash marks) is considered to be impervious to moisture transfer. The top boundary (no hash marks) is considered to be at a constant moisture content throughout the sorption. This assumes that the surface of the food instantaneously equlibrates to its equilibrium moisture content upon contact with the atmosphere. This would result in an area of large concentration gradient (rapid change in moisture content) and could cause an overprediction in the initial stages of the sorption prediction (Segerlind, 1976). To compensate for this, the elements closest to the boundary were made smaller and increased in size as the center was approached. The radius of each of the foods was 50 mm and thickness of the samples is given in Table 2.

The average moisture contents for shredded wheat, cookies, nonfat dried milk and ground beef as predicted by Finite Element model are compared with the experimental values in Fig. 4. Each of the foods seem to show a good fit to the predicted values of the Finite Element model. Two of the foods, the flour at 0.11 a_w and the freeze-dried turnip (Fig. 5) showed an overprediction of the moisture content as compared to experimental values when diffusivity is assumed to be constant.

The mass transfer parameters for the raisin are also shown in Table 1. This product was also modeled as a finite
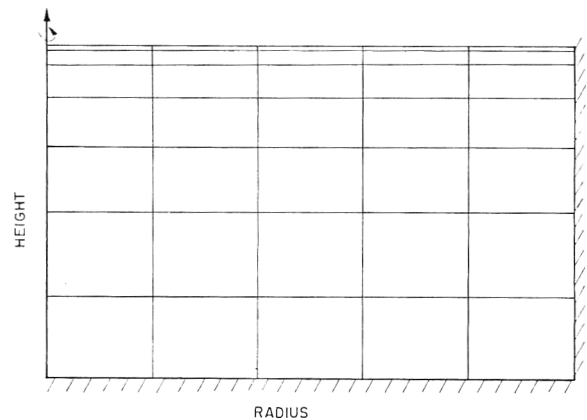
Fig. 3—Finite Element representation for sorption in a petri dish, 35 4-node isoparametric elements.

Table 2—Goodness of fit (mean relative deviation modulus, P) for model predictions

Product	Ambient air a_w	Sample thickness mm	P value Constant diffusivity	P value Variable diffusivity
Flour	0.11	3.6	7.95	1.48
Flour	0.75	5.2	3.2	
Flour	0.75	7.9	1.03	
Flour	0.75	14.3	1.61	
Flour	0.75	a	1.49	
Nonfat dry milk	0.75	3.2	1.03	
Freeze-dried apple	0.75	2.7	1.07	
Freeze-dried turnip	0.75	2.5	1.83	1.55
Freeze-dried raw ground beef	0.75	10.9	2.04	
Oatmeal cookie	0.75	10.1	1.29	
Shredded Wheat	0.75	2.9	0.52	
Raisin	0.75	b	7.55	

ᵃ Flour in a pyramid shape.
ᵇ Cylinder shape, length = 14.5 mm; radius = 4.1 mm.
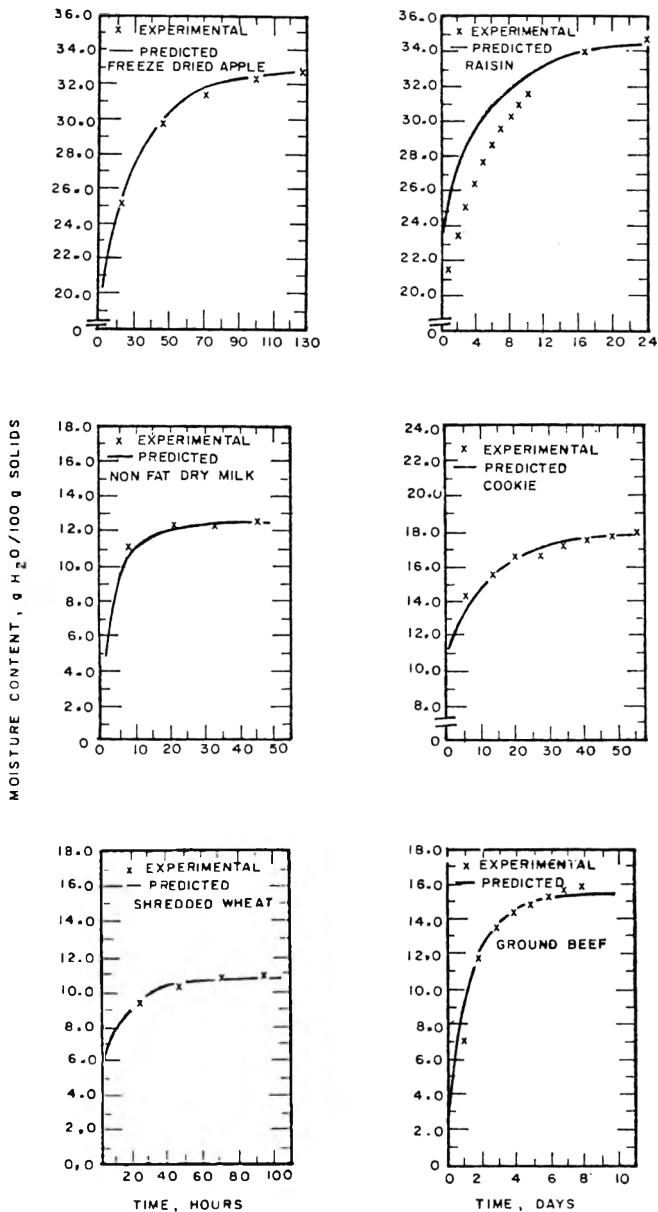
Fig. 4—Comparison of experimental data to model predictions for different foods adsorbing to 0.75 a_w.
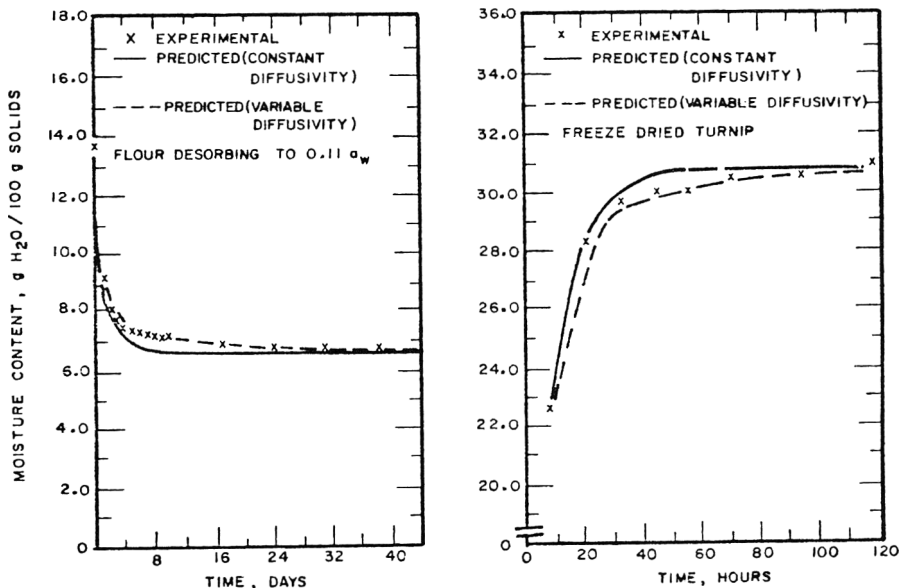
cylinder, axisymmetric; however, due to the symmetry involved, half of the total solid was modeled. The raisin was divided into 49 4-node isoparametric elements. Moisture transfer was assumed to take place through the ends as well as from the radial surface. Again to compensate for the large gradients along the surface boundaries, smaller elements were utilized along the surface and increased as the center is approached. The Finite Element prediction is given in Fig. 4. As can be seen, an overprediction is observed throughout the majority of the adsorption. This may have occurred for several reasons. Many of the raisins were actually elliptical in shape and were not well described as cylinders. In many cases the raisins were flattened on one side, making that side impervious to moisture transfer, which would in turn cause an overprediction by the Finite Element model.

For comparison purposes, the mean relative deviation modulus, P (Eq. 6) was calculated for each of the predictions:

$$P = (100/n) \sum_{i=1}^{n} \frac{M_i - Mp_i}{M_i} \qquad (6)$$

where n is the total number of data points, M_i is the actual data point, and Mp_i is the predicted moisture content.

Table 2 lists the P values obtained for each of the foods. As can be seen from the table, the majority of the predictions had P values less than 2.0. The largest deviation was for the raisin, P = 7.55, followed by the flour at 0.11 a_w, freeze-dried raw beef and freeze-dried turnip. Based on the predictions (Fig. 5) and larger P-values for the freeze-dried turnips and the flour at 0.11 a_w, these products were re-evaluated using variable diffusivities.

Products represented by a variable diffusivity

Table 1 lists the two food products exhibiting a variable diffusivity, flour at 0.11 a_w and turnip. Variable diffusivities were selected based on the plots of ln Γ versus time (Γ is the moisture ratio). The plots for these food products were found to have a break at Γ = 0.12 for flour of 0.11 a_w and Γ = 0.05 for the freeze-dried turnip. A Deff for each of the portions of the curve was calculated. The Deffs calculated from these plots were called (Deff)1 and (Deff)2 and are shown in Table 1.

The Finite Element predictions for both the variable and constant diffusion coefficient cases are given in Fig. 5. The figure indicates that the predictions improved with a

Fig. 5—Comparison of experimental data to model predictions for foods described by variable diffusivity.

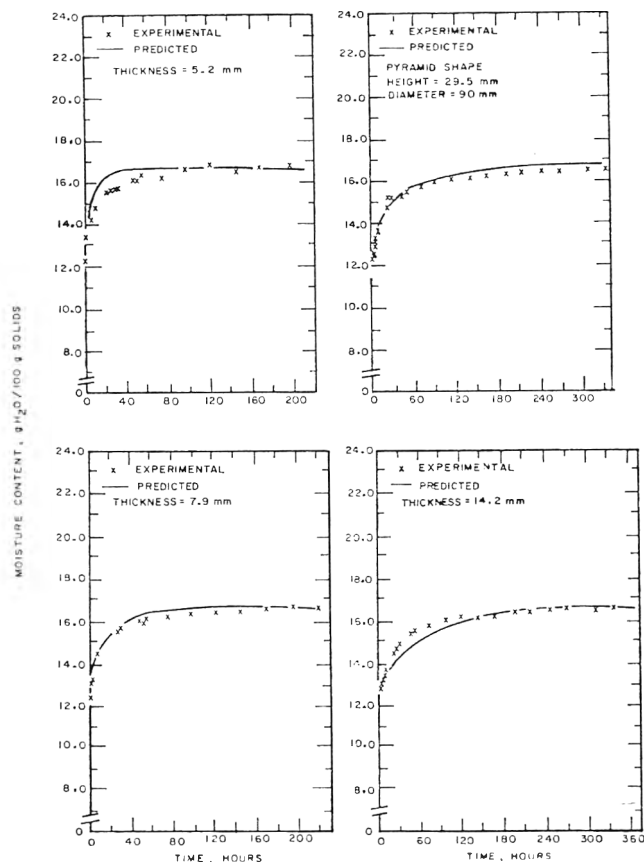Fig. 6—Comparison of experimental data to model prediction for flour with different thicknesses and shape.

Fig. 7—Finite Element representation of flour in a pyramid shape, 51 4-node isoparametric elements.

variable diffusivity. P values for each of the two foods are given in Table 2. In both cases the P values were better for the variable diffusivity than for the constant diffusivity case.

Verification of the finite element model for different thickness and shape

In the preceding section, the Finite Element predictions were carried out for a finite cylinder with one thickness each. In this section, the model will be validated for different cylinder heights and a pyramid shape. The flour sample adsorbing to 0.75 a_w was utilized for this purpose. The following three thicknesses were evaluated: 5.2, 7.9, and 14.2 mm. The pyramid shape had a height of 29.5 mm and a 90 mm diameter at the base. The Finite Element predictions are shown by thickness in Fig. 6. This figure shows that the best prediction was for the intermediate thickness, 7.9 mm. An overprediction is seen for the thinnest sample, 5.2 mm, and an underpredicton for the thicker sample, 14.2 mm. This is probably due to the averaging of the diffusion coefficients obtained for each thickness. The middle thickness sample had a Deff closest to the average Deff and showed the best fit.

A final prediction was made for the flour in a pyramid shape. The Finite Element representation is given in Fig. 7. The figure represents all of the food and contains 51 4-

node isoparametric elements, again with the boundaries represented by small elements increasing in size towards the center of the product to overcome the moisture gradients. The Finite Element prediction is given in Fig. 7. The figure shows a good prediction relative to the experimental data, with a slight overprediction in the latter portions of sorption. Table 2 lists the P values for the flour with different thicknesses. The majority are less than 2.0 except for the flour at 5.18 mm which has a P = 3.20.

In general, the Finite Element model shows an adequate prediction for the moisture content for foods representing different groups, sizes and shapes. This model can be used as an aid in predicting equilibrium time for a food in a given environment, ingredient mixing and other behavior of stored foods. With modifications to include the temperature dependence of the parameters, the model could be used as an aid in shelf life predictions during a distribution cycle (relative humidity and temperature fluctuating). and temperature fluctuating).

REFERENCES

Crank, J. 1975. "The Mathematics of Diffusion," 2nd ed. Oxford University Press, London.
Fischer, K. 1935. New methods for the quantitative determination of water in fluids and solids. Agnew. Chem. 48: 394.
Lomauro, C.J., Bakshi, A.S., and Labuza, T.P. 1985. Moisture transfer properties of dry and semimoist foods. J. Food Sci. 50: 397.
Misra, R.N. and Young, J.H. 1980. Numerical solution of simultaneous moisture and shrinkage during soybean drying. Trans. ASAE 23: 1277.
Naveh, D. 1983. Analysis of transient conduction heat transfer in the thermal processing of foods using the Finite Element method. Ph.D. thesis, Univ. of Minnesota, St. Paul, MN.
Naveh, D., Kopelman, I.J., and Pflug, I.J. 1983. The Finite Element method in thermal processing of foods. J. Food Sci. 48: 1086.
Norrie, D.H. and DeVires, G. 1978. "An Introduction to Finite Element Analysis." Academic Press, New York.
Polivka, R.M. and Wilson, E.L. 1976. Finite Element Analysis of Nonlinear Heat Transfer Problems. Report No. VCSESM 76-2. Dept. of Civil Eng., Univ. of California, Berkeley, CA.
Segerlind, L.J. 1976. "Applied Finite Element Analysis." John Wiley and Sons, New York.
Zhang, T., Bakshi, A.S., Gustafson, R.J., and Lund, D.B. 1984. Finite Element analysis of nonlinear water diffusion during rice soaking. J. Food Sci. 49: 246.
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Moisture Transfer Properties of Dry and Semimoist Foods

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————————ABSTRACT————————

Equilibrium moisture content, mass diffusion coefficients, and densities were collected for wheat flour, shredded wheat, raisins, nonfat dry milk, and freeze-dried apple, turnip, and ground beef at 25 ± 1°C. Flour adsorbing to 0.75 a_w had the largest mass diffusion coefficient, 1.15×10^{-7} m²/hr and raisins had the smallest value of 0.015×10^{-7} m²/hr. Two of the food products, flour desorbing to 0.11 a_w and the freeze-dried turnip, exhibited diffusion coefficients which were dependent on the moisture content. Most of the foods reached equilibrium within 1 wk based on an objective criterion of no more than a 0.5% dry basis moisture difference over three successive readings at 1 wk interval.

INTRODUCTION

ONE OF THE BASIC physical phenomena occurring in foods during processing and storage is water transport. This could be diffusion of water inside the food or moisture exchange between the food and the environment. The amount of water present at any location in the food affects characteristics such as drying time and shelf life. With the introduction of high-speed computers and powerful numerical techniques, e.g., finite element analysis, it has become feasible to develop models describing water transport phenomena. These models can aid in the reduction of the number and type of experiments required to understand the influence of environmental factors and types of packaging on food quality during processing and storage. The parameters necessary to predict the moisture content with time using a mathematical model are moisture diffusion coefficient, equilibrium moisture content and surface mass transfer coefficient. In most of the sorption modeling studies, it is assumed that the surface of food product reaches equilibrium instantaneously. This is necessary because of the lack of availability of mass transfer prediction equations for the food products exposed to almost zero air velocity. Thus, the diffusion coefficients calculated based on this assumption may be viewed as effective diffusion coefficient which account for both internal and external resistance to moisture transfer.

Various methods have been cited in the literature to calculate diffusion coefficients. One method of calculation is to determine the Deff as a function of the material properties of the food and its surrounding atmosphere (King, 1968; Bluestein, 1971; Roman et al., 1982). Other methods include solving numerically Fick's second law of diffusion (Eq. 1) and then finding the value of Deff which minimizes the error in sum of squares between the actual and predicted values of the moisture content (Zhang et al., 1984; Bakshi and Singh, 1980; Misra and Young, 1980; Steffe and Singh, 1980; Whitaker and Young, 1972).

$$F = -D \frac{\partial c}{\partial x} \qquad (1)$$

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A third method is to use an analytical solution to Eq. (1) such as the one given below for a moisture transfer in one dimension in a semi-infinite slab:

$$\Gamma_{is} = \frac{M - Me}{Mo - Me} = \frac{8}{(\Pi)^2} \sum_{n=0}^{\infty} (2n+1)^{-2}$$

$$\exp \frac{-Deff(2n+1)^2(\Pi)^2 t}{4L^2} \qquad (2)$$

where Me = equilibrium moisture content; Mo = initial moisture content; M = moisture content at time t; L = thickness of the slab. Eq. (2) assumes a uniform initial moisture content, constant surface moisture content and a constant density. Hanson et al. (1971) and Roman et al. (1982) have used the following in determining effective or apparent diffusion coefficient:

$$Deff = -(4L^2/\Pi^2) * slope \qquad (3)$$

where the slope is obtained from a plot of ln Γ versus time. This method is especially useful when a break in the Γ vs time plot is observed. A Deff can be obtained for each portion of the curve.

Very little is said in the literature concerning the determination of an equilibrium moisture content in a given saturated atmosphere. A constant weight is generally implied by the term equilibrium moisture content (Neuber, 1980). Zuritz et al. (1979) suggested weighing the food samples until there was no weight change as measured to the second decimal place (0.01g). Ferrel et al. (1966) recorded weight change with time until a change in weight corresponding to less than 0.05% change in the moisture content was observed. Labuza (1984) suggests recording the change in weight until the change in moisture content is less than 0.001g water/g dry solids.

The specific objectives of this study were to: (1) To test an objective criterion for determining the equilibrium moisture content when sorption isotherms are prepared; (2) Determine the equilibrium moisture contents of foods representing different food groups at 25 ± 1°C and different relative humidities; (3) Determine the mass diffusivity of foods representing different food groups at 25 ± 1°C. In addition, the changes in density were monitored to quantify swelling or shrinkage and corresponding changes in dimension, if measurable.

MATERIALS & METHODS

ALL OF THE FOODS used in this study (Table 1) were purchased from a local supermarket. The foods were selected in an attempt to represent different food groups. The sorption experiments were carried out in desiccators and Mason jars at 25 ± 1°C, in the presence of still air. All of the foods were adsorbed to 0.75 a_w from their initial stage. The flour was both adsorbed and desorbed to 0.52 and 0.11 a_w, respectively. In addition the flour was adsorbed to 0.75 a_w for three thicknesses and one different shape. Prior to each sorption experiment, the moisture content was determined by the Karl Fischer method (Fischer, 1935) using an Aquatest IV titrator (Photovolt Corporation, New York, NY). Saturated salt solutions were used to generate a constant equilibrium relative humidity within the desiccators or Mason jars. The saturated salt solutions were

made according to the guidelines of the Hygrodynamics Technical Bulletin #5. The following salt solutions were used in this study: lithium chloride (0.11 a_w); magnesium nitrate (0.52 a_w), and sodium chloride (0.75 a_w).

During the sorption experiments all of the foods were held in petri dishes (plastic, 15 × 60 mm). The samples in the desiccators were weighed at 1-wk intervals. The weights were recorded to 0.00001 g (Mettler H51, Mettler Instrument Corporation, Princeton, NJ) as a function of time. Moisture content was converted to a dry basis (g H_2O/g solids). Equilibrium was believed to be reached when the moisture content (dry basis, g water/g solids) did not change by more than 0.5% for three consecutive sampling periods of not less than 7 days. The equilibrium moisture content was considered to be the first in the series of consecutive readings. The samples were left in the Mason jars for six months.

To collect more data points for calculating diffusion coefficients, sorption experiments were also repeated in the desiccators. The sampling frequencies ranged from 12 – 24 hr for the foods in the desiccators. An additional experiment was conducted for flour adsorbing to 0.75 a_w as follows: A fish tank with the saturated salt solution was modified so a food sample in a petri dish could be suspended from the top. This allowed monitoring of the sample weight without removing the food from the equilibrium atmosphere.

The solid density of the food samples was determined immediately prior to and following the sorption experiments. An air comparison pycnometer (Beckman Model 930, Beckman Instruments, Inc., Fullerton, CA) was utilized for the density determinations.

Deff was found for each of the foods in this study using Eq. (2) and a nonlinear optimization technique on a computer. The first 60 terms in Eq. (2) were used for calculating the value of deff. A small study was conducted to determine the minimum number of terms so that the series solution given by Eq. (2) coverages. This number was found to be 60.

RESULTS & DISCUSSION

Equilibrium moisture content

If the uptake of moisture by a food product is internally controlled by diffusion, theoretically an infinite time is

Table 1—Products used in sorption experiments

Food Group	Product
Cereal	Flour
Dairy	Nonfat Dry Milk
Fruit	Freeze Dried Apple
Vegetable	Freeze Dried Turnip
Meat	Freeze Dried Ground Beef
Semi-Moist Bakery Product	Oatmeal Cookie
Formulated Cereal Product	Shredced Wheat
Dense/Slow Sorbing Product	Raisin

required to reach equilibrium. Fig. 1 shows an example of flour (in a 3 mm thick layer) adsorbing to a a_w of 0.75. The flour sample, shown in Fig. 1, does not show a constant moisture content even after 10 days storage period. The fluctuations in moisture content may be due to disturbances caused by the equilibrium atmosphere, temperature fluctuations of the room and/or the error in determining the sample weight itself.

Equilibrium was believed to have been reached when the moisture content (dry basis, g water/g solids) did not change by more than 0.5% during three consecutive sampling periods at not more than 7 day intervals. A change in 0.5% in the moisture content represents a weight change of about 1 mg/g, which equals the accuracy of a typical balance found in food analysis laboratory. The equilibrium moisture content was considered to be the first in the series of the three consecutive readings. This objective criterion for equilibrium moisture content was compared to the values obtained after 6 months storage in Mason jars. A tabulated comparison is given in Table 2. The range of difference between the equilibrium moisture content as determined by the 6-month study and objective criterion was −0.51g water/100g solids for shredded wheat adsorbing to 0.52 a_w to +0.77g water/100g solids for the freeze-dried apple. If the values of the moisture content after 6 months are assumed to be true or closer to the true equilibrium content, a percent error can be calculated. The percent error was generally less than 5%. Even though some of
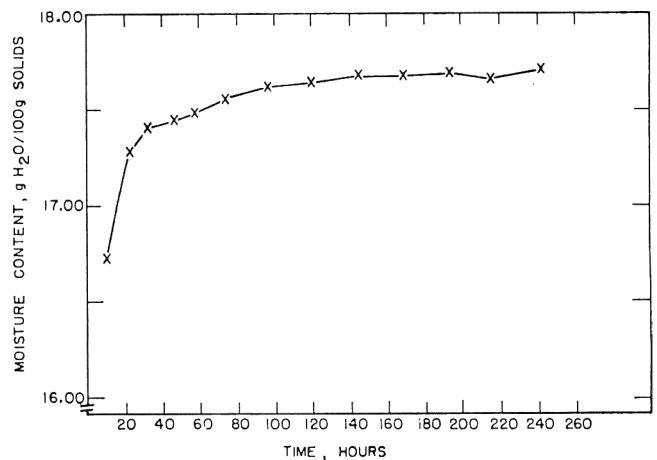
Fig. 1—Typical adsorption curve for food products used in this study. (Flour adsorption, 0.75 a_w, 3 mm thickness).

Table 2—Equilibrium moisture contents of foods used in sorption experiments

Product	a_w	Equilibrium moisture content (g water/100g solids)		% Error	Difference[c] (g water/ 100g solids)
		Mason jar[a]	Desiccator[b]		
Flour	0.11	5.94 (0.09)	6.28 (0.07)	5.7	−0.34
Flour	0.52	13.27 (0.10)	13.26 (0.11)	0.1	+0.01
Flour	0.75	mold	16.87 (0.08)	—	—
Nonfat dry milk	0.75	12.59 (0.01)	12.73 (0.17)	1.1	−0.14
Freeze-dried apple	0.75	34.07 (0.39)	33.30 (0.66)	2.3	+0.77
Freeze-dried turnip	0.75	28.12 (0.59)	mold	—	—
Freeze-dried raw ground beef	0.75	12.62 (0.39)	12.68 (0.40)	0.5	−0.06
Oatmeal cookie	0.75	17.97 (0.05)	17.76 (0.06)	1.2	+0.21
Shredded Wheat	0.75	14.98 (0.09)	15.49 (0.19)	3.4	−0.51
Raisin	0.75	mold	mold	—	—

[a] Six month Mason jar experiment, average of three replicates, number in ( ) is the standard deviation.
[b] Desiccator experiments, average of five replicates, equilibrium moisture content evaluated by 0.5% criterion, number in ( ) is the standard deviation.
[c] The absolute difference between the value of the moisture content from the Mason jar and desiccator.

the foods had become moldy after the 6-month period and were not compared, the same general trends can be expected to hold. As seen in Table 2, after the 6-month period both nonfat dry milk and shredded wheat showed a slightly lower equilibrium moisture content than the value obtained from the desiccators. For the nonfat dried milk, this may be due to an amorphous-to-crystalline change of the sugar present during long term storage which would reduce moisture content (Saltmarch and Labuza, 1980). For the shredded wheat, a similar type of crystallization of the amylose or amylopectin may also have occurred causing expulsion of the adsorbed water. Differences may also be due to weighing errors.

In general, the 0.5% criterion seems to be satisfactory with sampling for a weight change at 7-day intervals. The 0.5% criterion was used for all of the remainder of the sorption experiments.

Table 3—Solid density of foods used in sorption experiments

Product	a_w	(g Solids/cc food)[a]	
		Initial	Final
Flour	0.11	1.28 (0.015)	1.37 (0.020)
Flour	0.52	1.28 (0.015)	1.41 (0.021)
Flour	0.75	1.28 (0.015)	1.19 (0.018)
Nonfat dry milk	0.75	1.31 (0.017)	1.21 (0.014)
Freeze-dried apple	0.75	1.31 (0.018)	1.21 (0.014)
Freeze-dried turnip	0.75	1.02 (0.019)	1.12 (0.016)
Freeze-dried raw ground beef	0.75	1.14 (0.021)	1.09 (0.022)
Oatmeal cookie	0.75	1.29 (0.016)	1.09 (0.019)
Shredded Wheat	0.75	1.30 (0.107)	1.20 (0.019)
Raisin	0.75	1.23 (0.018)	1.07 (0.018)

[a] Average of three replicates. Number in ( ) is the standard deviation.

Density

The solid density was determined for each food product before and after the sorption experiments. The results of the density determinations are given in Table 3. The majority of the foods follow the general trend of a decrease in density with adsorption and an increase in density with desorption. The standard deviation of the density as measured by air comparison pycnometer was 0.065 g/cc. Considering this and a small observed change in density after absorption or desorption, a negligible change in volume of food product can be assumed.

Diffusion coefficients

The effective diffusion coefficient, Deff, calculated for each product takes into account the effects of a variable diffusion coefficient, variable heat of adsorption, particle nonuniformity, shape irregularity, swelling and other nonideal behavior (Hanson et al., 1971). Deffs were calculated for each food product assuming the product was represented as an infinite slab for most of the foods, except for the raisin which was assumed to be a finite cylinder. Eq. (2) was used for the slab calculations and Eq. (2), (4), and (5) were utilized for the raisin calculations. All of the calculations were carried out using the first 60 terms of the infinite series solution.

$$\Gamma_{ic} = \frac{M - Me}{Mo - Me} = \frac{4}{a^2} \sum_{n=1}^{\infty} \left(\frac{1}{bn^2} \right) \exp(-Dbn^2 t) \quad (4)$$

where bn = roots of $Jo(bn \times a) = 0$; $Jo(bn \times a)$ = Bessel function of first kind of order zero; a = radius of the cylinder; Γ_{ic} = moisture ratio of semi infinite cylinder.

$$\Gamma\text{finite cylinder} = \Gamma_{is} \times \Gamma_{ic} \quad (5)$$

Plots were made of ln Γ vs time. Fig. 2 shows a typical curve found for the majority of the foods in this study. There is a linear relationship between ln Γ and time. The
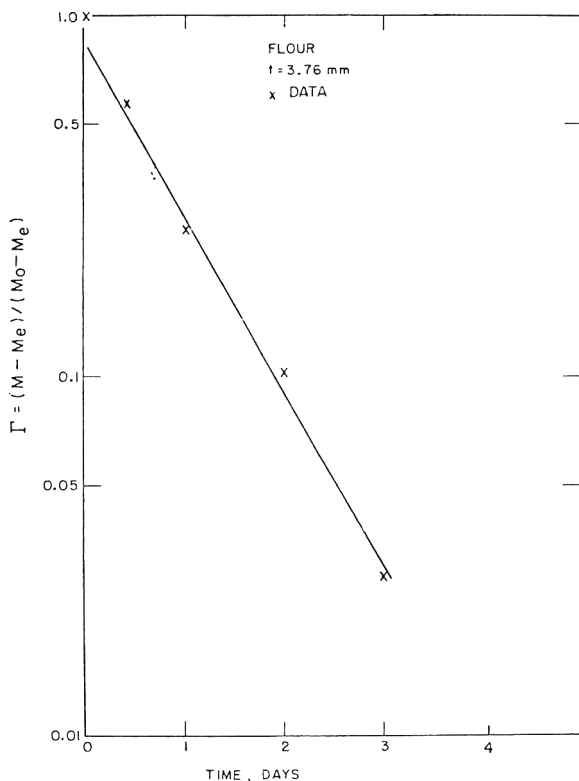

Fig. 2—Moisture ratio versus time plots for food products characterized by a constant diffusion coefficient, exemplified by flour adsorbing to 0.75 a_w.
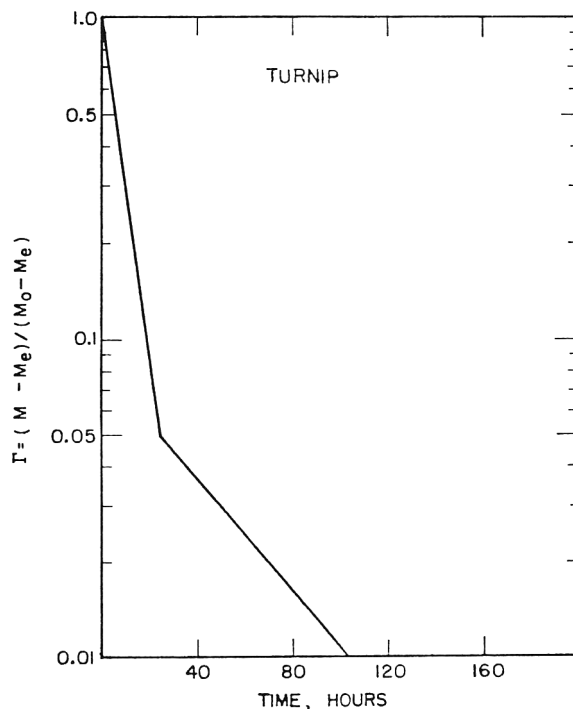

Fig. 3—Moisture ratio versus time plots for food products characterized by a variable diffusion coefficient, exemplified by freeze-dried turnip adsorbing to 9.75 a_w.

Table 4—Effective diffusion coefficients of foods used in sorption experiments (5 replicates per meausrement)

Product	a_w	Thickness (mm)	Initial weight (g)	Diffusion coefficient[a] $m^2/hr \times 10^7$
Flour	0.11	3.6	4.0	0.139[b] (0.006)
Flour	0.75	8.3	28.3	1.152 (0.826)
Nonfat dry milk	0.75	3.1	2.0	0.767 (—)
Freeze-dried apple	0.75	2.7	0.9	0.146 (0.015)
Freeze-dried turnip	0.75	2.5	0.5	0.274[c] (0.009)
Freeze-dried raw ground beef	0.75	10.9	6.2	1.105 (0.212)
Oatmeal cookie	0.75	10.1	12.8	0.143 (0.015)
Shredded Wheat	0.75	2.9	2.0	0.199 (0.026)
Raisin	0.75	d	0.5	0.015 (0.006)

[a] Deff in m^2/hr, number in ( ) is the standard deviation.
[b] (Deff)1 = 0.146 (0.009); (Deff)2 = 0.022 (0.001).
[c] (Deff)1 = 0.268 (0.003); (Deff)2 = 0.050 (0.003).
[d] Cylinder shape, length = 14.5 mm; radius = 4.1 mm.

flour of 0.11 a_w and the freeze-dried turnip both showed breaks in the plot of ln Γ versus time. A representative plot is shown in Fig. 3 for the freeze-dried turnip. The breaks were observed at $\Gamma = 0.12$ for the flour at 0.11 a_w and $\Gamma = 0.05$ for the freeze-dried turnip. As a result, a Deff for each portion of the curve was calculated. The resulting values were termed (Deff)1 and (Deff)2. The adsorption of flour to 0.52 a_w resulted in the reaching of equilibrium within a 12 hr period. As a result of the limited number of points, Deff was not calculated.

Labuza (1984) stated that the Deff for water in air is about $9.4 \times 10^{-2} m^2/hr$. The Deff for moisture diffusion in a porous solid generally ranges from $3.6 \times 10^{-10} m^2/hr$ for a dense food such as a raisin to $3.6 \times 10^{-5} m^2$ for most freeze-dried foods (Labuza, 1984). The range of Deff found in this study was found to be $1.15 \times 10^{-7} m^2/hr$ to $1.5 \times 10^{-9} m^2/hr$, the values for each of the products are found in Table 4. Several observations can be made from Table 4. The raisin exhibited the smallest Deff, $1.5 \times 10^{-9} m^2/hr$. This could be due to the lack of porous structure internal to the raisins as compared to the other food. The Deff for adsorption (flour, 0.75 a_w), $1.15 \times 10^{-7} m^2/hr$ is much greater than the value for desorption (flour, 0.11 a_w), $1.39 \times 10^{-8} m^2/hr$. Hanson et al. (1971) found similar results for adsorption and desorption of moisture on granular corn starch. They attributed this to the drying of the outer portion of the starch granule, causing a lower Deff for that surface. This results in the formation of a barrier of high mass transfer resistance to the water molecules in the interior of the granule. This drying of the outer surface during desorption may also have occurred for flour. Sample compaction and nonuniformity of the sample thickness may also have led to discrepancies in the values of Deff. The two observed Deffs for the freeze dried turnip may be due to the following: an initial swelling of the product followed by a relaxation of the porous structure resulting in a total collapse in structure causing a lower overall Deff in the final stages of sorption. This is only a hypothesis and additional work is needed to varify this.

The Deff for the flour adsorbing to 0.75 a_w was calculated from an average of three thicknesses ranging from 5.5 – 14.2 mm. The equilibrium moisture content was calculated from the thinnest sample, 5.5 mm. The flour adsorbing to 0.75 a_w had the largest standard deviation, 0.826. This could be due to the different thicknesses used. The heat of sorption could also have accounted for a larger diffusion coefficient, especially for the thicker sample. The analysis suggested by King (1968) can be used to determine the change in moisture transfer coefficients due to heat transfer effects. However, this could not be done in the case of flour because of the unavailabity of the physical parameters needed and also the assumption of moisture transfer due to vapor diffusion by King (1968). The diffusion coefficients given in this paper are calculated based on the assumption that moisture transfer is due to liquid diffusion only.

CONCLUSIONS

AN OBJECTIVE CRITERION of no more than 0.5% dry basis moisture difference over three successive readings taken at one week intervals seems satisfactory for evaluating equilibrium moisture content of dry and semimoist food. Most of the foods reached equilibrium within 1 month at 25°C using the above criterion and the values compared well with the 6-month study. The equilibrium moisture content values for these foods are tabulated. The mass diffusion coefficients of the foods were determined to be in the range 1.15×10^{-7} to $1.15 \times 10^{-9} m^2/hr$. All the foods except flour desorbing to 0.11 a_w and freeze-dried turnips adsorbing to 0.75 a_w were adequately described by a constant diffusion coefficient. The diffusion coefficients of flour are different for adsorption and desorption.

REFERENCES

Bakshi, A.S. and Singh, R.P. 1980. Kinetics of water diffusion and starch gelatinization during rice parboiling. J. Food Sci. 45: 1387.
Bluestein, P.M. 1971. The kinetics of water vapor sorption in freeze dried foods. Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge, MA.
Crank, J. 1975. "The Mathematics of Diffusion," 2nd ed. Oxford University Press, London.
Ferrel, R.E., Shepard, A.D., Thelking, R., and Pence, J.W. 1966. Moisture equilibrium of bulgur. Cereal Chem. 43: 136.
Fischer, K. 1935. New methods for the quantitative determination of water in fluids and solids. Agnew. Chem. 48: 394.
Hanson, T.P., Cramer, W.D., Abraham, W.H., and Lancaster, E.B. 1971. Rates of absorption in granular corn starch. Chem. Eng. Prog. Symp. Series #108. 67: 35.
Hygrodynamics, Inc. Creating and maintaining humidities by salt solutions. Hygrodynamics Tech. Bull. 5, Silver Spring, MD.
King, C.J. 1968. Rates of moisture sorption and desorption in porous, dried foodstuffs. Food Technol. 22: 165.
Labuza, T.P. 1984. "Moisture Sorption: Practical Aspects of Isotherm Measurement and Use." The American Association of Cereal Chemists, St. Paul, MN.
Misra, R.N. and Young, J.H. 1980. Numerical solution of simultaneous moisture and shrinkage during soybean drying. Trans. ASAE 23: 1277.
Neuber, E. 1980. Critical considerations of moisture sorption isotherms exemplified by the sorption equilibria in corn. In "Proceedings of the Second International Conference on Physical Properties of Agricultural Materials and Their Influence on Technological Process." Commission International du Genie Rural IV/2 Vol. 5, Paper #143.
Roman, G.N., Urbicain, M.J., and Rotstein, E. 1982. Moisture equilibrium in apples at several temperatures: experimental data and theoretical considerations. J. Food Sci. 47: 1484.
Saltmarch, M. and Labuza, T.P. 1980. Influence of relative humidity on the physicochemical state of lactose in spray dried sweet whey powders. J. Food Sci. 45: 1231.
Steffe, J.F. and Singh, R.P. 1980. Liquid diffusivity of rough rice components. Trans ASAE 23: 767.
Whitaker, T.B. and Young, J.H. 1972. Simulation of moisture movement in peanut kernels: evaluation of the diffusion equation. Trans. ASAE 15: 163.
Zhang, T., Bakshi, A.S., Gustafson, R.J., and Lund, D.B. 1984. Finite element analysis of non-linear water diffusion during rice soaking. J. Food Sci. 49: 246.
Zuritz, C., Singh, R.P., Moini, S.M., and Henderson, S.M. 1979. Desorption isotherms of rough rice from 10C to 40C. Trans. ASAE 22: 433.
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Effects of Polymers on Secondary Nucleation of Ice Crystals

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——————ABSTRACT——————

The kinetics of ice crystallization in various polymer solutions were studied by the thermal response method in a batch crystallizer. Polymers suppress the secondary nucleation of ice crystals, depending on the kinds of polymer and concentration. The decrease of the nucleation rate constant in polymer solutions was related to the increase of viscosity. The effects of polymers on the nucleation rate were also observed in solutions containing low molecular weight compounds.

INTRODUCTION

THE PRINCIPLE, freeze concentration is a superior method for the concentration of liquid foods. Freeze concentration has become popular in the coffee or juice industries. Despite the many merits of freeze concentration, its utilization in industries is still limited, mainly due to the high capital cost and lack of reliability for the apparatus. In 1979 Thijssen postulated that freeze concentration systems would be more popular if the cost for dewatering by this method could be reduced by a half. Fundamental studies on freeze concentration are insufficient. Especially, information about ice crystallization in solutions containing high molecular weight substances is lacking. Ice crystallization is important in these systems because most liquid foods contain high molecular weight substances like proteins or polysaccharides as well as low molecular weight ones.

In the field of cryobiology, polymers are well known as cryoprotectants. Ahmed et al. (1975) reported that glycoproteins in the blood of polar fish work as antifreeze agents. Even synthetic polymers such as polyethylene glycol suppress the nucleation of ice (Michelmore and Franks 1982; Allegretto et al., 1982). However, these studies are limited to the homogeneous nucleation of ice. Although Omran and King (1974) showed that pectin suppressed the secondary nucleation of ice crystals, which occurs in the presence of seed crystals at low supercooling, there are no studies on secondary nucleation of ice crystals in polymer solutions. The object of this paper is to investigate the ice crystallization in solution containing various high molecular weight substances by thermal response methods in a batch crystallizer.

MATERIALS & METHODS

Samples

Solution of the following high molecular weight substances at concentration of 0.5 – 12.2% were used as samples. Dextran T2000 (M.W.:2,000,000 daltons), Dextran T500 (M.W.:500,000 daltons), Dextran T40 (M.W.:40,000 daltons), Ficoll 400 (M.W.:400,000 daltons), Ficoll 70 (M.W.:70,000 daltons) and dextran sulphate (M.W.:500,000 daltons) were purchased from Pharmacia Fine Chemicals. Crude ovalbumin, carboxy methylcellulose (CMC) and polyethylene glycol (M.W.:20,000 daltons) were obtained from Wako Pure Chemical Industries. A protein fraction of skim milk was

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separated by gel chromatography (Sephadex G-50). Ovalbumin was dialyzed before use. The molecular weight of CMC was determined as 10,000 daltons by measuring the viscosity.

Theory

When a seed crystal is introduced into a solution of which supercooling has been kept constant adiabatically, a thermal response curve shown in Fig. 1 is obtained (Omran and King 1974).

The induction time method (ITM) developed by Omran and King (1974) and Stocking and King (1976) and the supersaturation time curve method (STCM) developed by Kane et al. (1974) and extended by Shirai et al. (1985) are useful for extracting the kinetics of ice crystallization from the temperature response curve.

In the ITM, the information of kinetic parameter is obtained from an induction time, t_c necessary for a slight temperature rise δ (0.0018°C in this study) in region I. Stocking and King (1976) proposed the following equation under the assumption that the nucleation rate was proportional to the i th power of the supercooling and the growth rate was proportional to the supercooling and inversely proportional to the radius of the ice particles:

$$\delta = \frac{4\sqrt{2}\lambda\rho_I kvbg^{1.5}\Delta T^{\circ i+1.5}t_c^{2.5}}{5MCp} \tag{1}$$

where λ is the latent heat of ice; ρ_I, the density of ice; kv, the volume shape factor; b, the nucleation rate constant representing the effect of the seed characteristics, flow conditions etc.; g, the growth rate factor representing the dependency of the diffusivity and the thermal conductivity of the solution, flow conditions etc.; ΔT°, the initial supercooling; t_c, the time interval; M, the total mass in the crystallizer; and Cp, the specific heat capacity of the contents of crystallizer. Eq. (1) indicates that the increase of the time interval, t_c, means the decrease of the nucleation rate or the growth rate of ice crystals. However, these cannot be evaluated independently.

In the STCM the maximum slope on the temperature time curve was measured. Kane et al. (1974) proposed a method to calculate the change in temperature and particle size distribution of ice crystals during batch crystallization by solving the population balance equation with respect to particle size and the heat balance equation simultaneously under the assumption that the growth rate was independent of the crystal size, and deduced the kinetic parameter, β, which corresponds to the nucleation rate per crystal in continuous crystallizers and depends on the nucleation rate and the growth
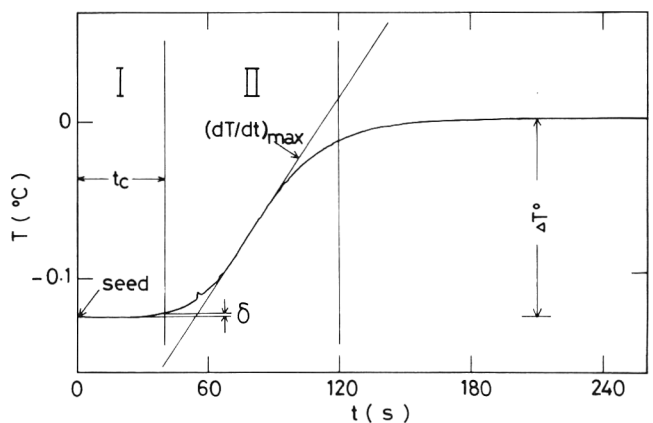

Fig. 1—Thermal response curve; 10% Dextran T500 solution; ΔT° = 0.130°C.

rate of ice crystals. They further correlated β with the initial supercooling and the maximum slope by computer simulation. The method for calculating the temperature response curve and the change in crystal size distribution during the course of batch crystallization was extended by Shirai et al. (1985) to the case of crystal size dependent growth rate. After extensive calculation, the relationship among the supercooling, the maximum slope and the kinetic parameter β was given by Eq. (2):

$$\beta^\circ \Delta T^\circ = 5.93(\mu_2^\circ)^{0.027} (dT/dt)_{max} \qquad (2)$$

where μ_2° is the initial second moment of crystal size distribution, and β°, the initial value of β. The parameter β was expressed by an implicit increasing function of the growth rate constants g_{a0} and g_{a-1} and the nucleation rate constant a_n defined by Eq. (3) and (4), respectively, (Shirai et al., 1985):

$$G_a = g_{a0} + g_{a-1}/r \qquad (3)$$

$$\dot{N} = a_n \mu_n \qquad (4)$$

where G_a is the linear growth rate of the a axis of ice crystals; r, the radius of the ice particles; $\dot{N}$, the nucleation rate of ice crystals
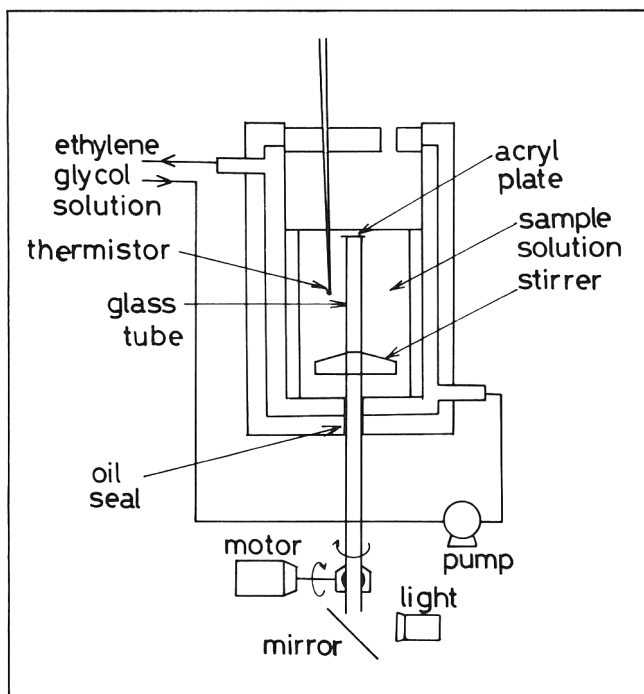
Fig. 2—Schematic diagram of the crystallizer used.
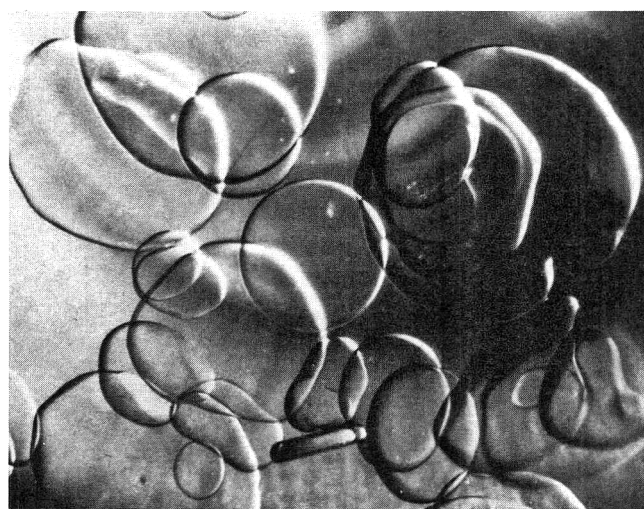
per unit volume; and μ_n the n th moment of ice crystal size distribution. The values of g_{a0} and g_{a-1} are determined by the Nusselt number or Sherwood number correlation for agitated vessels proposed by Huige and Thijssen (1972). By assuming that the nucleation rate is proportional to the second power of the supercooling and to the second moment of the crystal size distribution, the nucleation rate constant is written as Eq. (5):

$$a_2 = A_3 \Delta T^2 \qquad (5)$$

The coefficient of the nucleation rate constant, A_3, is determined from β° and calculated values of g_{a0} and g_{a-1}.

MATERIALS & METHODS

THE EXPERIMENTAL set-up and procedures were the same as those of our previous work (Shirai et al. 1985) except the method for observation of ice crystals by a traveling microscope. Fig. 2 shows the schematic diagram of the crystallizer used. A light source for the microscope was placed beneath a long agitating shaft made of a glass tube (7 mm o.d.) with an acryl plate end. The thickness of the space between the surface of the sample solution and the acryl plate was about as thin as 1mm in order to supply enough light even in solutions like skim milk. A seed crystal 3 μL was introduced into the solution in which supercooling had been kept constant, and the temperature change caused by the release of latent heat with ice crystal growth was measured by a thermistor (Ishizuka denshi 512 CT). After the temperature reached equilibrium, agitation was stopped, and ice crystals floating in the space above the acryl plate of the agitating shaft were observed with the microscope and photographed with a camera with 30-fold magnification. The viscosity of polymer solutions were measured with a B type rotational viscometer (Tokyo Keiki Seisakusho), with a cylinder of 19 mm diameter, at 60 rpm in 300 mL beakers at 0°C.

RESULTS & DISCUSSION

The shape of ice crystal formed in polymer solutions

Fig. 3 shows typical photographs of ice crystals formed in pure water (Fig. 3—left) and 1.5% dextran sulphate solution (Fig. 3—right). Transparent and disc shaped ice crystals were observed in pure water. The ice crystals formed in every polymer solution used in this study were also disc shape with small grains like bubbles radiating from the center of the ice crystals. The ratio of the height to the diameter of ice crystals formed not only in pure water but in every polymer solution was determined to be 1:8 to 1:7.

Kinetic parameter β

The kinetic parameter β was determined by the STCM. Fig. 4 shows the relationship between β and initial super-
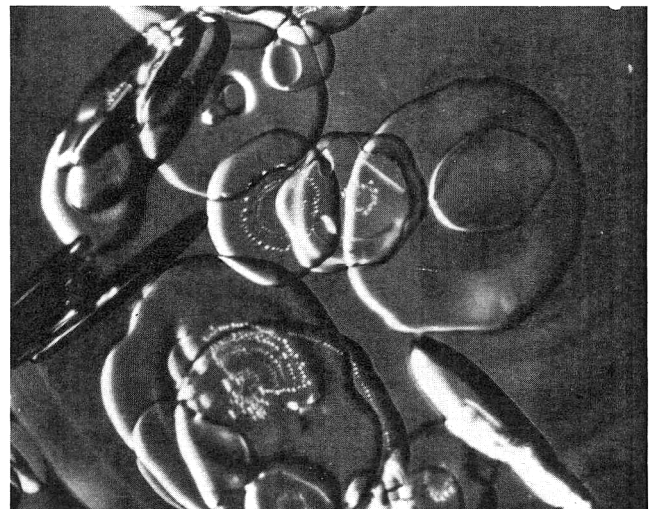
Fig. 3—Ice crystals formed in pure water; $\Delta T^\circ = 0.116°C$ (left) and those in 1.5% dextran sulphate solution; $\Delta T^\circ = 0.098°C$ (right).

cooling (ΔT°) in the solutions tested. The values of β° in the polymer solutions increased with initial supercooling and had smaller values of β° than that in water at the same degree of supercooling. To confirm the reliability of the results, a statistical treatment was done using "Students' t". The values of $\ln \beta^\circ$, obtained from the results of Fig. 4 by the least square method at the initial supercooling of $0.05^\circ C$, are shown in Table 1 with the confidence coefficient, P, in per cent. P is the maximum confidence coefficient, below which the confidence intervals of $\ln \beta^\circ$ for each solution and water never overlap. The viscosity of each polymer solution is also shown in Table 1. The parameter β is expressed as the increasing function of the growth rate and the nucleation rate of ice crystals (Shirai et al., 1985), that is, the decrease of either the nucleation or growth rate reduces the β value. However, the growth rate would not decrease significantly with the addition of polymer, since little freezing point depression was observed and the decrease in the calculated heat transfer coefficient with an increase in viscosity was insignificant in the experimental range. Then the decrease in the β values caused by the polymers added is ascribable mainly to the decrease in nucleation rate.

Induction time t_c

Fig. 5 shows the relationship between the induction time, t_c, determined by the ITM, and initial supercooling (ΔT°) in the sample solutions. The values of t_c in polymer solutions decreased with increased initial supercooling, as did those in water. The statistical analysis of the $\ln t_c$ values is shown in Table 1. The low P values in ITM may be attributed to difficulty in the measurement of very small temperature changes. However, it seems that the $\ln t_c$ values are different from the result for water especially in the case of polymer solutions with high viscosity. Thus, the values of t_c in polymer solutions were inclined to be greater than in water at the same degree of supercooling. This result is ascribable to the decrease in the nucleation rate of ice crystals in polymer solutions with the same reason as discussed with respect to β, because the t_c is expressed as a decreasing function of the growth rate and the nucleation rate of ice crystals as shown in Eq. (1).

Mean ice crystal size

Taking into consideration the results of β and t_c, it is expected that the size of ice crystals will increase in polymer solutions. The mean radii of ice crystals, $\overline{r}$, in sample solutions are shown in Fig. 6. In Table 1 the $\overline{r}$ values are statistically tested. The P values are relatively high as a whole, though they scatter. This may be due to the difficulty in the measurement of ice crystal size especially in high supercooling, because the ice crystals are small and tend to agglomerate together. The observed ice crystals in polymer solutions were larger than those in water. Thus, the nucleation rate of ice crystals would decrease in the polymer solutions.
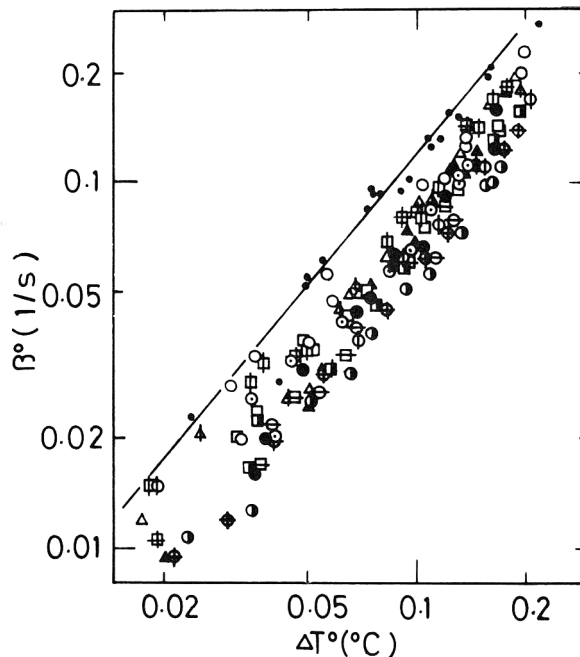
Fig. 4—Relationship between β° and ΔT° in polymer solutions. Keys: ○; 1% Dextran T500, ●; 5% Dextran T500, ◑; 10% Dextran T500, ◐; 5% Dextran T2000, ⊖; 0.5% CMC, ⊙; 1.5% dextran sulphate, ✦; 5% dextran sulphate, □; 12.2% protein fraction of skim milk, ⊕; 3% Dextran T40, ⊟; 10% Dextran T40, ◻; 5% polyehtylene glycol, ✧; 11.2% ovalbumin, △; 5% Ficoll 400, ▲; 10% Ficoll 400, ◭; 10% Ficoll 70, •; water. The line for water was obtained by the least square method with the correlation coefficient of 0.983.

Table 1—Statistical treatment of the $\ln \beta^\circ$, $\ln t_c$, and $\overline{r}$ values at the initial supercooling of $0.05 \,^\circ C^a$

Solution	Viscosity (kg/m s) × 10^2	$\ln \beta^\circ$	P (%)	$\ln t_c$	P (%)	$\overline{r}$ (μm)	P (%)
10% Dextran T500	3.15	−3.75 ± 0.31	99	4.64 ± 0.53	80	458 ± 75*	80
5% dextran sulphate	2.73	−3.64 ± 0.29	99	4.51 ± 0.36	80	437 ± 64*	90
0.5% CMC	2.35	−3.43 ± 0.28	70	4.13 ± 0.20	50	523 ± 40***	90
12.2% protein fraction of skim milk	1.7†	−3.54 ± 0.27	95	4.63 ± 0.48	80	n.m.	
5% Dextran T2000	1.44	−3.29 ± 0.20	60	4.38 ± 0.32	80	425 ± 68*	60
1.5% dextran sulphate	1.40	−3.30 ± 0.14	70	4.04 ± 0.10	60	471 ± 61	95
5% Dextran T500	1.25	−3.40 ± 0.13	95	4.14 ± 0.18	70	469 ± 52	99
5% polyethylene glycol	1.25	−3.37 ± 0.13	90	4.18 ± 0.20	70	360 ± 10	60
11.2% obalbumin	1.21	−3.38 ± 0.21	70	–		n.m.	
10% Ficoll 400	1.10	−3.55 ± 0.27	98	4.20 ± 0.21	70	n.m	
10% Dextran T40	0.90	−3.51 ± 0.28	95	4.27 ± 0.33	70	– **	
10% Ficoll 70	0.70	−3.27 ± 0.14	70	4.00 ± 0.07	60	n.m.	
5% Ficoll 400	0.67	−3.29 ± 0.15	70	4.04 ± 0.15	50	437 ± 55***	90
3% Dextran T40	0.50	−3.17 ± 0.11	50	–		394 ± 37*	60
1% Dextran T500	0.45	−3.18 ± 0.11	50	–		387 ± 33*	70
water	0.18	−2.97 ± 0.12t (18,α)		3.66 ± 0.27t (18,α)		329 ± 20t (18,α)	

a P: the maximum confidence coefficient below which the confidence intervals of the values of $\ln \beta^\circ$, $\ln t_c$ and $\overline{r}$ for each solution and water never overlap.
–: the difference of the values between the solutions and water could not be shown statistically. n.m.: not measured. t(18,α): Students 't with the degree of freedom of 18 and the significance level α (=100-P). The number of experiments: *; 4, **; 5, ***; 6; others; >7. †: determined by extrapolation.

Effects of viscosity of polymer solutions on nucleation rate

Using the coefficient of nucleation rate constant, A_3, determined by the method described in the section "Theory" the temperature time curve and the final crystal size distribution after equilibrium were calculated according to the method proposed by Shirai et al. (1985). Good agreements between calculated and experimental results for ice crystallization in water and 5% Dextran T500 solu-

tion (Shirai et al., 1985) suggested the appropriateness of the method to obtain A_3. Fig. 7 shows the relationship between the coefficient of the nucleation rate constant, A_3, and the viscosities of polymer solutions. The line in Fig. 7 was determined by the least square method using all the A_3 values calculated from the experimental data. The points in Fig. 7 are determined by averaging the A_3 values obtained for each polymer solution. Judging from the statistical analysis, it was found that a linear correlation with a slope of -0.0336 ± 0.0078 between the ln A_3 values and the viscosities with the confidence level of 95% exists. Although polymer solutions might behave as non-Newtonian fluids, the measured viscosities were accepted as representatives in polymer solutions. In the polymer solutions, the nucleation rate decreases with the increase in viscosity of the polymer solutions as shown in Fig. 7.
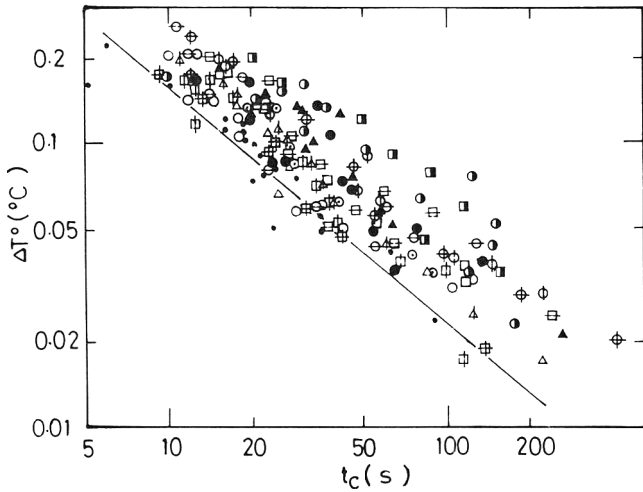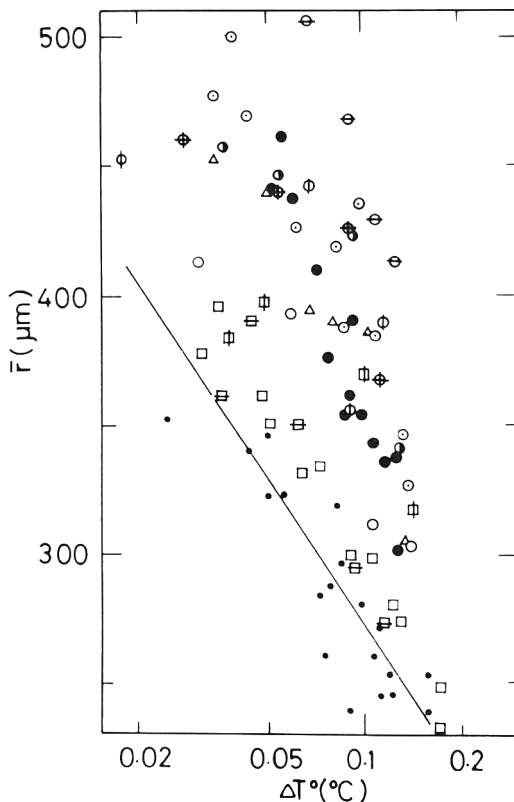
Yagi and Yoshida (1975) observed the behavior of gas bubbles in the agitating vessel and found that a mass of gas surrounded the impeller like a doughnut in sodium polyacrylate solutions and CMC solutions. They ascribed the phenomenon to the normal stress effect (Weissenberg effect), that is, the radial outward liquid flow from the impeller is restricted because of the high viscosities of solutions. If this phenomenon occurs in crystallizers with an increase in viscosity, the frequency of collisions between ice crystals and wall or impeller decreases and consequently the nucleation rate may decrease.

Contrary to this macroscopic effect, Michelmore and Franks (1982) ascribed the inhibition of homogeneous nucleation of ice by polyethylene glycol to a perturbation of the diffusional freedom of water molecules by the polymer. Although it is not clear whether this concept is acceptable or not in our secondary nucleation system, other microscopic factors (for example, molecular weight, shape of molecules, and interaction among molecules) might contribute to the decrease of the secondary nucleation as well as macroscopic factors.

Franks et al. (1983) inferred an increase in the interfacial free energy between ice and an aqueous phase of solutions of hydroxyethyl starch through homogeneous nucleation experiments with a differential scanning calorimetric method. If the addition of polymer used in this study increases the interfacial free energy, it acts as an inhibitor of secondary nucleation of ice crystals, since nuclei born from a seed crystal, which would be able to survive in water, might melt before they grow because of high interfacial free energy.

Fig. 5—Relationship between t_c and $\triangle T^\circ$ in polymer solutions. Keys are the same as those in Fig. 4. The line for water was obtained by the least square method with the correlation coefficient of -0.934.

Fig. 6—Mean radius of ice crystals formed in polymer solutions. Keys are the same as those in Fig. 4. The line for water was obtained by the least square method with the correlation coefficient of -0.904.

Fig. 7—Relationship between the coefficient of nucleation rate constant and viscosity of polymer solutions. The line was obtained by the least square method with the correlation coefficient of -0.48. The number of data is 232. The range of each point shows that the true value lies with the probability of 95%.
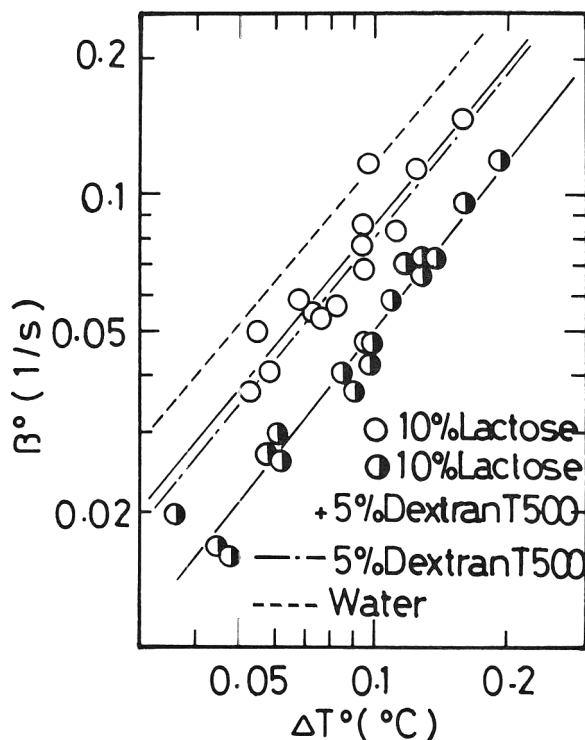
Fig. 8—Effect of Dextran T500 on lactose solution. The lines were obtained by the least square method with correlation coefficients of 0.923 and 0.977 for 10% lactose solution and the mixed solution, respectively.

Effects of polymer on the secondary nucleation in solution of low molecular weight compounds

Many foods, for example milk, consist of both low and high molecular weight substances. Fig. 8 shows the relationship between the initial β value and the initial supercooling for the solutions of each component and multicomponents. The mean size of ice crystals in the same sample solutions are shown in Fig. 9. The lines in Fig. 8 and Fig. 9 were obtained by the least square method. No linear correlation between $\bar{r}$ and $\Delta T°$ in the case of 10% lactose solution was found. Fig. 8 shows that the $\beta°$ values in 10% lactose solution are nearly the same as those in 5% Dextran T500 solution. Mean sizes of ice crystals in the dextran solution seem greater than in water while those in the lactose solution seem smaller. Therefore, the decrease of the β values in the lactose solution is due to the decrease of the growth rate of ice crystals, while in the dextran solution it is the nucleation rate.

In the mixed solution of lactose and dextran, the $\beta°$ values decrease still more because of the decrease of the growth rate and the nucleation rate of ice crystals in the solution. The mean sizes of ice crystals in the mixed solution would be greater than those in lactose solution, and they are smaller than those in 5% Dextran T500 solution. These results indicate that the nucleation rate decreases even in a lactose solution by the addition of high molecular weight compounds, and that the effects of each solute on the kinetic parameters would be independent in the mixed solution.

Finally the above phenomena were confirmed in a liquid food. Fig. 10 shows the mean sizes of ice crystals formed in 20% skim milk compared with 10% lactose solution. The skim milk contains about 10% lactose. The mean size of crystals in the skim milk is greater than that in the lactose solution. Protein in the skim milk would also contribute to the enlargement of the mean size of crystals.
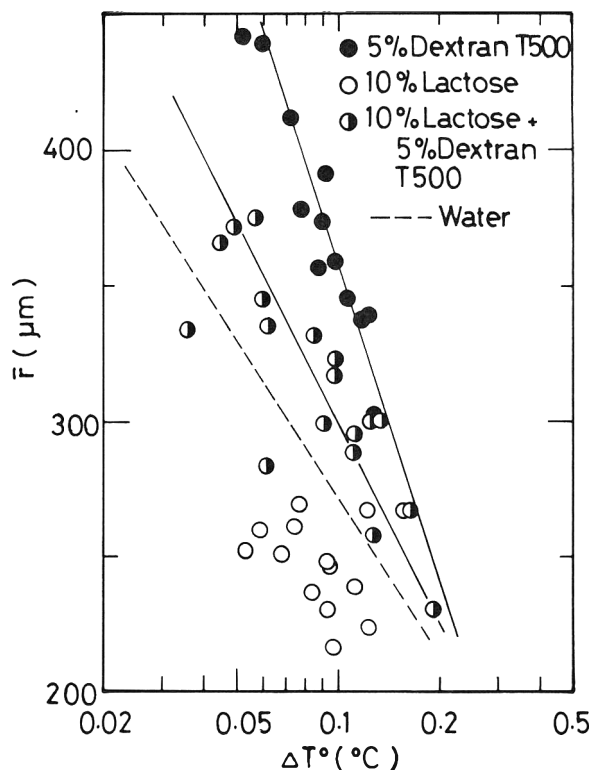
Fig. 9—Effect of Dextran T500 on mean radius of ice crystals formed in lactose solution. The lines were obtained by the least square method with the correlation coefficient of −0.811 for the mixed solution.
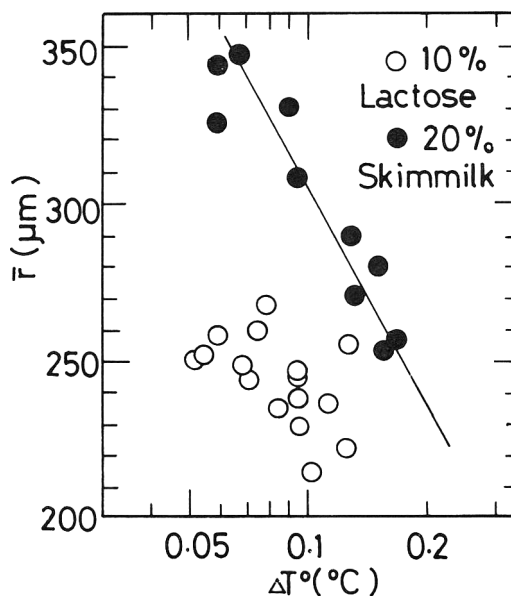
Fig. 10—Mean radius of ice crystals formed in 20% skim milk and 10% lactose solution. The line for skim milk was obtained by the least square method with the correlation coefficient of −0.921.

CONCLUSION

ICE CRYSTALLIZATION in various polymer solution was investigated in a batch crystallizer by STCM and ITM. The secondary nucleation of ice crystals was suppressed by the polymers depending on the viscosity of the solutions.

NOMENCLATURE

a_n = nucleation rate constant in Equation (4). $(1/s\ m^n)$

A_3 = coefficient of the nucleation rate constant in Equation (5). $(1/s\ m^2\ °C^2)$

b = nucleation rate constant in Equation (1). $(1/s\ °C^i)$

Cp = specific heat capacity of contents of crystallizer. $(kcal/kg\ °C)$

$(dT/dt)_{max}$ = maximum slope of thermal response curve. $(°c/s)$

G_a = growth rate parallel to the basal plane. (m/s)

g = growth rate factor in Equation (1). $(m^2/s\ °C)$

g_{a0} = growth rate constant in Equation (3). (m/s)

g_{a-1} = growth rate constant in Equation (3). (m^2/s)

kv = volume shape factor. $(-)$

M = total mass in the crystallizer. (kg)

N = actual nucleation rate. $(1/s\ m^3)$

r = radius of an ice crystal. (m)

$\bar{r}$ = mean radius of ice crystals. (m)

T = temperature. $(°C)$

t = time. (s)

t_c = induction time. (s)

Greek

β = kinetic parameter of ice crystallization; nucleation rate per crystal in a continuous crystallizer. $(1/s)$

β^o = initial value of β. (the values for the initial supercooling). $(1/s)$

ΔT = supercooling. $(°C)$

ΔT^o = initial supercooling. $(°C)$

δ = temperature rise in the induction time period. $(°C)$

ρ_I = density of ice. (kg/m^3)

μ = viscosity. $(kg/m\ s)$

μ_n = n th moment of crystal size distribution. (m^n/m^3)

μ_2^o = initial second moment of seed ice crystal. (m^2/m^3)

λ = latent heat of fusion of ice. $(kcal/kg)$

REFERENCES

Ahmed, A.I., Feeney, R.E., Osuga, D.T. and Yeh, Y. 1975. Antifreeze glycoproteins from antarctic fish. J. Biol. Chem. 250: 3344.

Allegretto, E.A., Caple, G., and Culbertoson, L.B. 1982. Inhibition of ice nucleation by synthetic polymers. ABS. PAP. ACS. 183: 19.

Franks, F., Mathias, S.F., Parsonage, P., and Tang, B.T. 1983. Differential scanning calorimetric study on ice nucleation in water and in aqueous solutions of hydroxyethyl starch. Thermoc. Acta. 61: 195.

Huige, N.J.J. and Thijssen, H.A.C. 1972. Production of large crystals by continuous ripening in a stirrer tank. J. Crystal Growth 13/14: 483.

Kane, S.G., Evans, T.W., Brian, P.L.T., and Sarofim, A.F. 1974. Determination of the kinetics of secondary nucleation in batch crystallizers. AIChE J. 20: 855.

Michelmore, R.W. and Franks, F. 1982. Nucleation rate of ice in undercooled water and aqueous solutions of polyethylene glycol. Cryobiology 19: 163.

Omran, A.M. and King, C.J. 1974. Kinetics of ice crystallization in sugar solutions and fruit juices. AIChE J. 20: 795.

Shirai, Y., Nakanishi, K., Matsuno, R., and Kamikubo, T. 1985. On the kinetics of ice crystallization in batch crystallizers. AIChE J. (In press).

Stocking, J.H. and King, C.J. 1976. Secondary nucleation of ice in sugar solutions and fruit juices. AIChE J. 22: 131.

Thijssen, A.H.C. 1979. The food industry in a changing society. Chem. Eng. Prog. 75: 21.

Yagi, H. and Yoshida, F. 1975. Gas absorption by Newtonian and non-Newtonian fluids in sparged agitated vessles. Ind. Eng. Chem. Proc. D.D. 14: 488.

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Effect of Heat Treatment on Bioavailability of Meat and Hemoglobin Iron Fed to Anemic Rats

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─────────── ABSTRACT ───────────

Efficiency of converting dietary iron from meat, bovine hemoglobin (HB) and ferrous sulfate into hemoglobin was investigated in anemic rats. Raw or autoclaved HB, and raw, autoclaved, boiled, or baked beef round, and ferrous sulfate were mixed into diets to give 36 mg Fe/kg diet. Heat treatments increased the efficiency of converting both HB and meat iron into hemoglobin by the anemic rats. Efficiencies of conversion were 23, 30, 33, 37, 37, 36 or 60 (LSD 0.05/0.01 = 4/6), respectively, for raw HB, autoclaved HB, raw meat, autoclaved meat, boiled meat, baked meat or ferrous sulfate. An in vitro measurement of iron availability in meat correlated poorly with bioavailability determined in rats. Cooking did not significantly affect the bioavailability of meat iron.

INTRODUCTION

THE DIGESTIBILITY and nutritive value of protein has long been known to be affected by heat processing. However, meat also is a major source of dietary iron in the U.S.A. Thermal processing of meat changes the oxidative state of its iron (Schricker and Miller, 1983; Lee and Shimaoka, 1984) and alters iron bioavailability. Autoclaving (121°C for 15 min) increased the relative biological value of sodium ferric pyrophosphate but did not affect the bioavailability of ferrous sulfate or ferric orthophosphate (Wood et al., 1978; Theuer et al., 1973). Boiling of intrinsically labelled rabbit hemoglobin for 3 min decreased the intestinal absorption of hemoglobin from 11 to 7% and 22 to 12% in normal and iron-deficient human subjects (Callender et al., 1957). Similarly, autoclaving meat at 121°C for 90 min was found to decrease the apparent iron absorption values when fed to weanling rats (McLaughlin, 1981; Tso, 1979). On the other hand, Turnbull et al. (1962) reported that cooking hemoglobin in a boiling water bath for 15 min did not alter the absorption of the hemoglobin iron in iron-deficient human subjects. Oldham (1941) also found that the hemoglobin gain in rats fed with oven-dried meat was higher than those fed with unheated, vacuum dried meat.

Since the results of the effect of heat processing are inconsistent, this experiment was done to study the effect of heat treatment on meat and hemoglobin iron bioavailability in anemic rats as influenced by the heme, nonheme and soluble iron components in meat and hemoglobin.

MATERIALS & METHODS

Diet preparation. Five portions of 1500g each of commercial ground beef round were prepared raw; boiled for 5, 30 or 90 min; or autoclaved at 120°C for 90 min. Three intact 1500-g portions from the same round were baked to internal temperature of 60° (rare). 70°(medium) or 80°C (well done), then ground. The meat was lyophilized, reground and analyzed for total iron, heme iron, crude protein, crude fat and phosphorus contents.

Total iron content was analyzed in ashed samples by using NO₃/acetylene flame atomic absorption spectrophotometry (Instrumentation Lab.,

Model 457, Wilmington, MA) at 248.3 nm (Boline and Shrenk, 1977; Clegg et al., 1981). Heme iron content was determined by extracting the heme pigments in the sample with acid acetone and then measuring the optical density at 640 nm (Hornsey, 1956). The protein and fat were determined by proximate analysis (Smith, 1980; Pearson, 1976; AOAC, 1975). The phosphorus content was determined by the vanado-molybdate colorimetric method (Pearson, 1976).

Bovine hemoglobin substrate powder type II (Sigma Chemical Company, St. Louis, MO), either raw or autoclaved at 121°C for 90 min, was analyzed for total iron and heme iron contents.

The composition of the diets used is shown in Table 1. The diets were individually mixed in 1.5 kg batches in a Twin Shell Dry Blender (Patterson-Kelly Co., East Stroudsbury, PA). Each diet was then analyzed for total iron, heme iron and soluble iron contents.

In vitro digestion. The amount of soluble iron in the diets was determined by incubating the dietary sample in 15 mL 0.1N HCl containing 1.5 mg pepsin for 3 hr at 37°C, on a shaker platform (Lab line, Melrose Park, IL) at setting 100. The mixture was then neutralized with 0.5N NaOH and then 7.5 mL 0.2M borate buffer (pH 8.0) containing 4 mg pancreatin and 0.05M sodium azide was added. The mixture was shaken at 37°C for another 24 hr and then centrifuged at 20,000 × g for 5 min. The solids were washed three times by resuspension of the pellet in 10 ml deionized water, followed by recentrifugation. The supernatant and washings were combined and adjusted to 100 mL. Iron content in the solution was determined by atomic absorption spectrophometry (Nelson and Potter, 1980; Lee and Clydesdale, 1979).

Animal repletion studies. Eighty weanling male Sprague-Dawley rats (Simonsen Laboratories, Gilroy, Ca) were housed individually in stainless steel metabolic cages and were provided with deionized water and diet ad libitum. They were maintained in a temperature controlled environment with a 12-hr light-dark cycle. They were made anemic by feeding the low iron basal diet (Table 1) for 7 days, and by removing approximately 0.7 mL blood twice, on the second and fourth day, from the retro-ocular

Table 1—Composition of diets containing iron from meat, hemoglobin and ferrous sulfate.

Ingredients (g)	Meat	Iron Sources Hemoglobin	FeSO₄	Basal[b] diet
Meat (lyophilized)	313	0	0	0
Hemoglobin[a]	0	109	0	0
FeSO₄·7H₂0	0	0	0.175	0
Vitamin free casein[c]	0	294	294	294
Rendered kidney fat[c]	52	70	70	70
Alpha-cellulose	50	50	50	50
NaH₂PO₄[c]	20	25	25	25
CaCO₃	15	15	15	15
Mineral mixture[d]	12	12	12	12
Vitamin mixture[e]	20	20	20	20
Dextrose	518	405	513.825	514

[a] Hemoglobin (bovine) substrate powder type II, Sigma Chemical Co., St. Louis, MO.

[b] Same composition as the diet containing 100% iron from ferrous sulfate, but no ferrous sulfate was added.

[c] Casein, rendered kidney fat and NaH₂PO₄ were supplemented to provide the same quantities of protein, fat and phosphorus as provided by meat diet (100g of lyophilized meat containing 11.2 mg Fe; 86.8g protein; 5.8g fat and 0.415g phosphate).

[d] Contained the following (g/kg): KCl 296.7; MgCO₃ 121.0; MnSO₄ 12.7; CoCl₂.6H₂0 0.7; CuSO₄.7H₂0 1.6; KI 0.8; Na₂MoO₄.2H₂0 0.1; ZnSO₄.7H₂0 28.0 and glucose to equal 1 kg.

[e] From Nutrition Biochemicals Corp., Cleveland, Ohio. It contained (g/kg): vitamin A concentrate (200,000 IU retinyl acetate/g) 4.5, vitamin D concentrate (400,000 IU calciferol/g) 0.25, niacin 4.5, riboflavin 1.0, pyridoxine HCl 1.0g, thiamin HCl 1.0, ascorbic acid 45.0, Ca pantothenate 30.0, biotin 0.02, folic acid 0.09, vitamin B₁₂ 0.00135 and dextrose to equal 1 kg.

capillary bed using a heparinized capillary tube. On day eight, body weight, hematocrit and hemoglobin concentration were determined for each rat. Hemoglobin concentration was measured by the cyanmethemoglobin method (Crosby et al., 1954).

These animals were then assigned to 8 groups of 10 animals each so that mean hemoglobin concentrations and mean body weights were similar among groups. About 10 grams of the experimental diets were fed daily to each rat for 10 days. One group of animals was selected as the low iron control which was continued on the basal diet throughout the repletion period. The total amounts of the diets consumed by each rat were determined by weighing diet given, refused and spilled.

On day ten of the repletion period, body weight, hematocrit and hemoglobin concentrations were again determined. The rats were then killed by decapitation and their livers removed, weighed and analyzed for iron content by atomic absorption spectrophotometry (Boline and Schrenk, 1977; Clegg et al., 1981).

Effeciency of converting dietary iron into hemoglobin (Regeneration Efficiency) was calculated as previously described (Mahoney and Hendricks, 1982; Mahoney et al., 1974). All feces from each rat were collected for the last 7 days of the repletion period. The feces were analyzed for total iron content by ashing overnight at 550°C, solubilizing the ash with hot 6N HCl and diluting appropriately with demineralyzed water for atomic absorption spectrophotometry. Apparent iron absorption expressed as percent, was calculated as [(iron intake minus fecal iron excretion) divided by iron intake] times 100.

Statistical analyses. Results were analyzed statistically by factorial analysis of variance (Cochran and Cox, 1957). Means were compared by the least significant difference (LSD) test (Ostle and Mensing, 1975) when "F" was statistically significant (Carma and Swanson, 1973).

RESULTS & DISCUSSION

THE TOTAL and heme iron contents of lyophilizied meat and diets are presented in Table 2. Fifty eight percent of the iron in ground beef was found as heme iron which is similar to the findings of Schicker et al. (1982) and Field et al. (1980). Heat treatment reduced the amount of heme iron in beef and bovine hemoglobin in a dose responsive manner. This is similar to the findings of others (Schricker et al., 1982; Schricker and Miller, 1983) for cooked meat. Heating blood (Schricker et al., 1982) or meat pigments (Chen et al., 1984) causes a modest increase in nonheme iron. The soluble iron contents in heat-treated meat diets were lower than that in raw meat diet but the difference was not statistically significant between raw and autoclaved bovine hemoglobin diets.

The animal responses to dietary iron treatment are presented in Table 3. Ferrous sulfate was used as the reference source of iron in this experiment. The regeneration efficiency for ferrous sulfate was the highest for all dietary treatments. This value is similar to those reported earlier from this laboratory (Park et al., 1983a,b).

Cooking meat did not affect iron bioavailability compared with uncooked product (Table 3). This was true whether bioavailability was expressed as regeneration efficiency, apparent absorption or liver iron concentration. The decrease in heme iron in meat due to cooking (Table 2) was not associated with any decrease in bioavailability of meat iron as fed to rats. In contrast Bogunjoko et al. (1983) reported that cooking decreased the bioavailability of iron from chicken muscle. The difference between their results and those in Table 3 may be methodological. They used an in

vivo rat gut segment technique and measured 59-iron uptake over a 2-hr period which may be inadequate for complete digestion of the meat protein compared with our 10-day rat feeding experiment. The regeneration efficiencies of 30.5 to 37.0% (Table 3) are similar to the 35% calculated from the data of Pye and McLeod (1946) but are lower than previously reported from this laboratory (Farmer et al., 1977; Mahoney et al., 1979).

Cooking improved slightly the bioavailability of hemoglobin iron (Table 3) even though its heme iron content was decreased (Table 2). Similarly, Park, et al. (1983a) found that cooking improved regeneration efficiency of fresh pork hemoglobin. Liver iron and iron regeneration efficiency were found to be significantly higher ($p < 0.01$) in rats fed ferrous sulfate diet than those fed meat or hemoglobin diets. This shows that, in anemic rats, the availability of iron from ferrous sulfate was higher than iron from meat or hemoglobin. The regeneration efficiency of ferrous sulfate diet was about two and three times the efficiencies of the meat hemoglobin diets. These results are in agreement with previous experiments (Amine and Hegsted, 1971; Bannerman, 1965; Wheby et al., 1970) in that the amount of ferrous sulfate iron absorbed by iron deficient rats was two to three times greater than the amount of dietary hemoglobin iron absorbed.

A simulated gastro-intestinal digestion using commercially available enzymes was performed to determine iron bioavailability. The soluble iron content thus determined was used as an indicator for iron bioavailability. A positive relationship between in vitro release of nonheme iron from vegetable foods and iron bioavailability has been reported by Narasinga-Roa and Prabhavathi (1978) and Nelson and Potter (1980). Amount of soluble iron in the diet, however, correlated poorly with the bioavailability of meat iron determined with rats (Fig. 1). The low amounts of soluble iron in this experiment may be due to the long incubation at neutral pH. Very insoluble hydroxides of iron could have been formed under these conditions of incubation resulting in the apparent low soluble iron values observed. Diets containing hemoglobin had more soluble iron than meat diets but hemoglobin diets had lower iron bioavailability in anemic rats than did meat diets. On the contrary, ferrous sulfate diet had higher soluble iron and also had high iron bioavailability. Even though the in vitro method is simple, rapid and low in cost, the results, with the exception of the ferrous sulfate diet, related poorly ($r = 0.63$) with bioavailability determined in the rats. Further research will be necessary before conclusions may be drawn on this subject.

REFERENCES

Amine, E.K., and Hegsted, D.M. 1971. Effect of diet on iron absorption in iron-deficient rats. J. Nutr. 101: 927.
AOAC. 1975. "Official Methods of Analysis," 11th ed. Association of Official Analytical Chemists, Washington, DC.
Bannerman, R.M. 1965. Quantitative aspects of hemoglobin-iron absorption. J. Lab. Clin. Med. 65: 944.
Bogunjoko, F.E., Neab, R.J., and Ledward, D.A. 1983. Availability of iron from chicken meat and liver given to rats. Br. J. Nutr. 50: 511.
Boline, D.R. and Schrenk, W.G. 1977. Atomic absorption spectroscopy of copper and iron in plant material. J. AOAC. 60: 1170.
Callender, S.T., Mallett, B.J., and Smith, M.D. 1957. Absorption of hemoglobin iron. Br. J. Haematol. 3: 186.
Carma, S.G. and Swanson, M.R. 1973. An evaluation of ten pairwise multiple comparison procedures by Monte Carlo methods. J. Am. Stat. Assoc. 68: 66.

Table 2—Heme and total iron contents of lyophilized meats, hemoglobin and diets (mg/kg).

| Iron source | Raw meat | Autoclaved[a] meat | Boiled meat | | | Baked meat | | | Hemoglobin[b] | | LSD[c] |
			5 min	30 min	90 min	Rare	Medium	Well done	Raw	Autoclaved	0.05/0.01
Lyophilized Product											
Total Iron	112	119	123	130	127	127	123	124	3220	3200	5/10
Heme iron	65	25	65.2	56.4	48.5	70.8	58.3	54.3	3200	1940	8/11
Diet											
Total iron	32.2	33.7	33.4	31.4	35.3	36.1	36.4	36.7	36.8	36.0	NS[d]
Heme iron	14.0	4.0	15.8	13.4	11.0	19.2	15.5	14.1	21.1	14.6	1.3/1.9
Soluble iron	9.2	8.2	0.99	4.7	5.2	4.6	4.9	5.7	2.0	21.5	NS

[a] Autoclaved 90 min at 121°C in glass canning jars.
[b] Hemoglobin data were not included in the LSD determinations because the data were not obtained during the same set of analyses.
[c] Mean differences must equal or exceed the least significant difference values to be statistically significant at the 5 or 1 percent levels of probability.
[d] Not statistically significant (P>0.05).

Table 3—Effect of dietary iron sources on hematinic response of anemic rats fed repletion diets for 10 days

Item	basal diet	FeSO$_4$ diet	Raw meat	Autoclaved[a] meat	Boiled meat			Baked meat			Hemoglobin		LSD[b] 0.05/0.01
					5 min	30 min	90 min	Rare	Medium	Well done	Raw	Autoclaved[a]	
Diet iron, mg/kg	5.1	39.5	32.2	33.7	33.4	31.4	35.3	36.1	36.4	36.7	36.8	36.0	—
Diet intake, g	80	96	107	99	105	99	105	106	109	110	87	92	12/15
Body weight, g					74								
Initial	72	74	72	72		72	72	72	73	74	72	72	NS[c]
Final	98	120	128	124	129	121	126	125	128	127	110	116	NS
Hemoglobin, g/dl													
Initial	6.00	5.90	5.93	5.87	5.84	6.21	6.31	6.02	6.03	6.07	5.87	5.93	NS
Final	4.82	12.17	7.35	7.86	7.06	7.63	8.45	8.33	8.45	8.66	6.79	7.50	1.00/1.33
HbFe gain/Fe intake, %	22.0	60.5	33.4	37.5	30.5	34.0	37.0	35.5	36.4	36.0	22.8	29.8	4.3/5.7
Absorption, %	61.6	87.7	54.0	53.0	58.4	51.3	62.5	48.8	60.8	59.1	55.2	58.7	6.8/9.1
Liver weight, g	3.20	4.75	4.25	4.37	4.42	4.18	4.51	4.22	4.67	4.38	4.10	4.48	0.43/0.57
Liver iron, μg/g	30.0	46.8	32.0	31.7	31.0	32.6	33.3	34.5	37.8	35.4	30.4	34.1	5.2/7.0

[a] Autoclaved 90 min at 121°C in glass canning jars.
[b] Mean differences must equal or exceed the least significant difference values to be statistically significant at the 5 or 1 percent levels of probability.
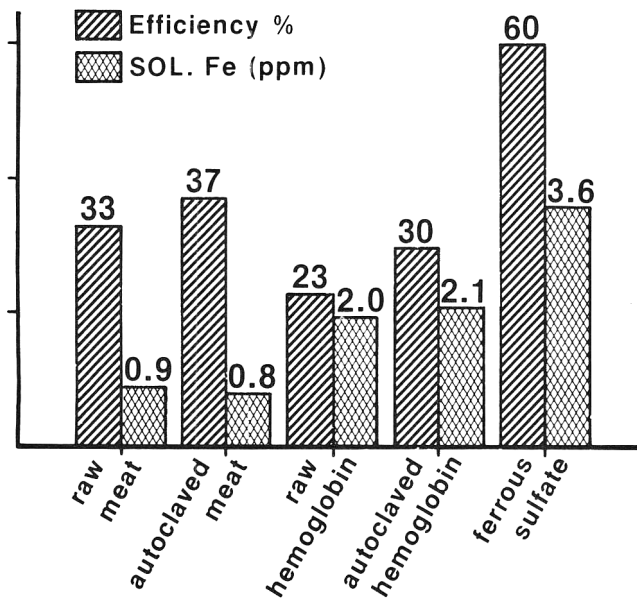[c] NS = Not statistically significant (P > 0.05).

Fig. 1—Efficiency of hemoglobin iron gain (regeneration efficiency) by anemic rats and soluble iron content of diets containing iron from raw or autoclaved meat, raw or autoclaved hemoglobin, or unheated ferrous sulfate.

Chen, C.C., Pearson, A.M., Gray, J.I., Fooladi, M.H., and Ku, P.K. 1984. Some factors influencing the nonheme iron content of meat and its implication in oxidation. J. Food Sci. 49: 581.

Clegg, M.S., Keen, C.L., Lonnerdal, B., and Hurley, L.S. 1981. Influence of ashing techniques on the analysis of trace elements in biological samples. 2. Dry ashing. Bio. Trace Element Res. 3: 327.

Cochran, W.G. and Cox, G.M. 1957. "Experimental Designs," 2nd ed. John Wiley and Sons, Inc., New York.

Crosby, W.H., Munn, S.I., and Furth, F.W. 1954. Standardized method for clinical hemoglobinometry. U.S. Armed Forces Med. J. 5: 693.

Farmer, B.R., Mahoney, A.W., Hendricks, D.G., and Gillett, T.A. 1977. Iron bioavailability of hand-deboned and mechanically deboned beef. J. Food Sci. 42: 1630.

Field, R.A., Sanchez, L.R., Kunsman, J.E., and Kruggel, W.G. 1980. Heme pigment content of bovine hemopoietic marrow and muscle. J. Food Sci. 45: 1109.

Hornsey, H.C. 1956. The colour of cooked cured pork. 1. Estimation of the nitric oxide-haem pigments. J. Sci. Food Agric. 7: 534.

Lee, K. and Clydesdale, F. M. 1979. Quantitative determination of the elemental, ferrous, ferric, soluble, and complexed iron in foods. J. Food Sci. 44: 549.

Lee, K. and Shimaoka, J. E. 1984. Forms of iron in meats cured with nitrite and erythorbate. J. Food Sci. 49:284.

Mahoney, A.W. and Hendricks, D.G. 1982. Efficiency of hemoglobin regeneration as a method of assessing iron bioavailability in food products. Pages 1-10 in C. Kies, ed. ACS symposium series 203. Nutrition bioavailability of iron. American Chemical Society, Washington, DC.

Mahoney, A.W., Hendricks, D.G., Gillett, T.A., Buck, D.R., and Miller, C.A. 1979. Effect of sodium nitrite on the bioavailability of meat iron for the anemic rat. J. Nutr. 109: 2182.

Mahoney, A.W., VanOrden, C.C., and Hendricks, D.G. 1974. Efficiency of converting food iron into hemoglobin by the anemic rat. Nutr. Metab. 17: 223.

McLaughlin, K. 1981. Bioavailability of calcium, fluoride and iron in mechanically-deboned beef fed to growing rats. M.S. thesis. Utah State University, Logan, Utah.

Narasinga-Roa, B.S. and Prabhavathi, T. 1978. An in vitro method for predicting the bioavailability of iron from foods. Am J. Clin. Nutr. 31: 169.

Nelson, K.J. and Potter, N.N. 1980. Iron availability from wheat gluten, soy isolate and casein complexes. J. Food Sci. 45: 54.

Oldham, H.G. 1941. The effect of heat on the availability of the iron of beef muscle. J. Nutr. 22: 197.

Ostle, B. and Mensing, R.W. 1975. "Statistic in Research," 3rd ed. The Iowa State University Press, Ames, IA.

Park, Y.W., Mahoney, A.W., Cornforth, D.P., Collinge, S.K., and Hendricks, D.G. 1983a. Bioavailability to anemic rats of iron from fresh, cooked or nitrosylated hemoglobin and myoglobin. J. Nutr. 113: 680.

Park, Y.W., Mahoney, A.W., and Hendricks, D.G. 1983b. Bioavailability of different sources of ferrous sulfate iron fed to anemic rats. J. Nutr. 113: 2223.

Pearson, D. 1976. "The Chemical Analysis of Foods," 7th ed. Chemical Publishing Co., Inc., New York, NY.

Pye, O.F. and Macleod, G. 1946. The utilization of iron from different foods by normal young rats. J. Nutr. 32: 677.

Schricker, B.R. and Miller, D.D. 1983. Effects of cooking and chemical treatment on heme and nonheme iron in meat. J. Food Sci. 48: 1340.

Schricker, B.R., Miller, D.D., and Stauffer, J.R. 1982. Measurement and content of nonheme and total iron in muscle. J. Food Sci. 47: 740.

Smith, V.R. 1980. A phenol-hypochlorite manual determination of ammonium nitrogen in Kjeldahl digests of plant tissue. Commun. in Soil Sci. Plant Anal. 11: 709.

Theuer, R.C., Martin, W.H., Wallander, J.F., and Sarett, H.R. 1973. Effect of processing on availability of iron salts in liquid infant formula products. Experimental milk-based formulas. J. Agric. Food Chem. 21: 482.

Turnbull, A., Cleton, F., and Finch, C.A. 1962. Iron absorption, IV. The absorption of hemoglobin iron. J. Clin. Invest. 41: 1897.

Tso, N. 1979. Effects of acid fermentation, dry-heat and wet-heat processing the bioavailability of calcium, iron and fluoride in mechanically-deboned beef. M.S. thesis. Utah State University, Logan, Utah.

Weintraub, L.R., Conrad, M.E., and Crosby, W.H. 1965. Absorption of hemoglobin iron by the rat. Proc. Soc. Exp. Biol. Med. 120: 840.

Wheby, M.S., Suttle, G.E., and Ford, III, K.T. 1970. Intestinal absorption of hemoglobin iron. Gastroenterology 58: 647.

Wood, R.J., Stake, P.E., Eisemen, J.H., Shippee, R.L., Wolski, K. E., and Koehn, U. 1978. Effects of heat and pressure processing on the relative biological value of selected dietary supplemental iron salts as determined by chick hemoglobin repletion assay. J. Nutr. 108: 1477.

Measuring Dietary Fiber in Human Foods

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————————ABSTRACT————————

Compositions of neutral detergent fiber (NDF) residues and water insoluble fiber fractions extracted by the Southgate method from five foods were determined quantitatively and compared. Neutral saccharide compositions of the two fiber residues, measured by HPLC, were similar for four of the five foods. Uronic acids constituted 4 – 8% of fiber in all foods. When adjustments were made for protein, starch and moisture contents, mean recovery of the two fibers was 97.9 ± 3.9%. Gravimetric quantitation of NDF or of a Southgate-derived insoluble fiber usually yielded a fiber content similar to the chemically determined value, although each of the Southgate residues had to be corrected for starch and/or crude protein content.

INTRODUCTION

MANY METHODS have been proposed to measure dietary fiber in human foodstuffs (Theander and Aman, 1979a, b; Anderson and Clydesdale, 1980; James and Theander, 1981). Typically, food samples are treated with solvents to remove lipids, soluble sugars and some pigments, and with an amylase to remove starch. Subsequently, samples are extracted with hot water to separate fiber into a water-soluble fraction containing gums, mucilages and variable proportions of the pectins and hemicelluloses, and a water-insoluble fraction consisting of cellulose, the rest of the hemicelluloses and pectins, and lignin (Asp and Johansson, 1981; Englyst, 1981; Furda, 1981; Schweizer and Wursch, 1981; Selvendran et al., 1981; Southgate, 1981; Theander and Aman, 1981). Many of these methods suffer from two weaknesses; they fail to include recoveries to check for fiber carbohydrate loss and they fail to remove starch adequately. The most well known and widely used method of dietary fiber analysis using this approach is the one by Southgate (1969). Developed to measure dietary fiber in human foods, it has been modified as a result of experience with the method (Southgate, 1976; Southgate, 1981). Many of the dietary fiber values for foods in the British food composition tables were obtained with this method (Paul and Southgate, 1978), although it is not generally appreciated that the accuracy of these fiber values are limited by contamination of the fiber residue with starch and by the use of colorimetric quantitation of an unknown mixture of sugars in the fiber acid hydrolysate. The neutral detergent fiber (NDF) method developed by Goering and Van Soest (1970) differs in that it recovers plant cell wall fiber as that fraction of a food insoluble in neutral detergent solution. Although originally developed to measure fiber in animal forages, it has been used to analyze human foods (Robertson and Van Soest, 1981). The modification of Schaller (1976), in which the residue is incubated with hog amylase, has been used to remove starch from the NDF residue, another problem in the original method. The NDF method is technically simpler and involves fewer manipulations than other methods. The criticism of this method that

it is gravimetric can be resolved if the NDF residue is acid hydrolyzed and subsequently analyzed for constituent neutral and acidic sugars and lignin (Slavin and Marlett, 1983; Neilson and Marlett, 1983).

The overall objective of this research was to evaluate and compare these two methods of fiber analysis. The compositions of the NDF residues and of the insoluble fiber fraction extracted by the Southgate method were determined quantitatively in five foods and compared. Secondly, samples were analyzed for nitrogen, starch and moisture to evaluate recoveries of the fiber residues and to determine the actual starch contamination of the residues. The third objective of the research was to compare the effectiveness of enzymatic starch removal from NDF and from the Southgate insoluble fiber fraction. The final objective was to evaluate the accuracy of the gravimetric determination of NDF by comparing the yield of this residue, corrected for starch and crude protein content when necessary, with the yield of NDF as determined by chemical analysis.

MATERIALS & METHODS

Sample preparation

Cereal and grain products were ground to 30 mesh in a Thomas-Wiley mill. Nationally available commercial grain products were selected for analysis except for the wheat bran (AACC-certified food grade wheat bran, St. Paul, MN); the coarse rolled wheat bread was produced by Holsum Bakeries, Inc. (Rice Lake, WI). The lima beans and peas (Del Monte), fresh apple (Washington State Red Delicious) and fresh carrots were procured locally, blended with distilled water and lyophilized. The dried apple pulp was a European Economic committee sample (James and Theander, 1981) (provided by Dr. H. Wiggins, MRC Dunn Nutrition Unit, Cambridge, England). All dried samples were stored at room temperature in sealed containers. Sample dry weights were determined on separate aliquots by AOAC procedures (1980).

NDF analysis

For chemical analysis, duplicate lyophilized NDF residues were prepared from five foods: wheat bran, lima beans, oatmeal, fresh apple and shredded wheat, using the Schaller modification (1976). Three different methods of removing starch from NDF residues were evaluated gravimetrically using 1 – 2g samples of 11 foods. Glass wool was used as a filtering aid (Marlett and Lee, 1980; Brauer et al., 1981). In the first modification dried food samples were incubated for 1 hr at 37°C with a bacterial amylase (E.C.3.2.1.1, from *Bacillus subtilus*, #A6505, Sigma Chemical Co., St. Louis, MO 63178) prior to refluxing in neutral detergent solution for 1 hr. In the second modification samples were refluxed in neutral detergent solution for 30 min, cooled and incubated at 37°C for 1 hr with the same enzyme (Robertson and Van Soest, 1981). Samples were then refluxed for another 30 min and the analysis completed according to the unmodified procedure (Marlett and Lee, 1980; Robertson and Van Soest, 1981). The third modification was the one proposed by Schaller (1976) in which prepared NDF residues are incubated with porcine amylase (Type VI-A, #A6880, Sigma). Preparation of all enzyme solutions and of the unmodified NDF residues has been described (Marlett and Lee, 1980; Robertson and Van Soest, 1981). Starch content of the NDF residues was determined enzymatically with amyloglucosidase (E.C.3.2.1.3, from A. Niger, #A3514, Sigma) (Brauer et al., 1981). Nitrogen was measured by Kjeldahl digestion and colorimetric quantitation (Weatherburn, 1967).

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Preparation of water-insoluble fiber residues

Water-insoluble fiber residues for chemical or gravimetric analysis were obtained essentially by the Southgate method (1969) as modified by Southgate (1976). Duplicate lyophilized food samples of 3 - 5g were treated and washed sequentially with hot methanol and hot diethyl ether (Southgate, 1976) under a hood and air dried overnight prior to pulverization with a mortar and pestle. Distilled water, 4 mL, was added to 200 - 300 mg aliquots of the extractive-free residue and sample placed in a boiling water bath for 10 min to gelatinize starch. Acetate buffer, 0.2 mL, 2M (Southgate, 1976), a 10% amyloglucosidase solution, 1 mL, (E.C.3.2.1.3, from Rhiszopus mold, #A7255, Sigma) and toluene were added and samples incubated overnight at 37°C. The enzyme was prepared by suspending 1g in 10 mL distilled water, centrifuging the sample at 3000 rpm for 10 min and discarding the pellet. Fiber polysaccharides were precipitated with four volumes (20 mL) 95% ethanol and washed with 10 mL 80% ethanol. The precipitate and supernatant were separated each time by centrifugation (Southgate, 1981). Residues were treated with 10 mL hot distilled water and placed in a boiling water bath for 20 min to extract the water-soluble fiber components (Southgate, 1981). The insoluble fiber residue was separated from the filtrate by centrifugation, the hot water extraction repeated and the sample recentrifuged. The gravimetric yield of dried insoluble residue was determined and aliquots representing 50 - 150 mg extractive-free residue taken for starch and nitrogen analysis, as described above.

Quantitative analysis of fiber residues

NDF residues were hydrolyzed by a modification of the Saeman et al. (1954) procedure in use in our laboratory (Slavin and Marlett, 1983). The procedure involves sequential 3 hr primary (72% sulfuric acid, ambient temperature) and 2 hr secondary (2N sulfuric acid, 100°C) acid hydrolysis steps and uses barium hydroxide for neutralization. Glass wool and any lignin were separated from the hydrolysate by vacuum filtration and the apparent lignin determined gravimetrically. Neutralized samples were lyophilized and dissolved in 5 mL distilled, deionized water. Excess barium ions were removed with a strong cation-exchange resin (AG 50W-X8, 200-400 mesh, hydrogen form, Bio-Rad Laboratories, Richmond, CA). Samples were filtered through a reverse-phase C_{18} resin (Sep-PAK, Waters Associates, Milford, MA) and through a 0.22 Millipore filter (Millipore Corp., Bedford, MA) (Slavin and Marlett, 1983). Aliquots, 50 mg, of the Southgate insoluble fiber fractions were hydrolyzed using the Blake and Richards (1970) modification of the Saeman procedure which uses a 1 hr primary (72% sulfuric acid) and a 3 hr secondary step (0.9 N sulfuric acid) and barium carbonate for neutralization (Neilson and Marlett, 1983). Following neutralization, ions and particulate matter were removed from the samples as described above. In all instances where insoluble material was present, the mixture was centrifuged at 1000 x g for 10 min and the resultant pellet washed free of acid with water, vacuum dried overnight at <40°C over P_2O_5, weighed and reported as apparent lignin (Neilson and Marlett, 1983).

The neutral sugar compositions of the hydrolysates were determined by HPLC using a carbohydrate analysis column (Aminex HPX-87P heavy metal, 300 mm x 7.8 mm, Bio-Rad) operated at 85°c with filtered and degassed water, at a flow rate of 0.6 mL/min (Neilson and Marlett, 1983). Galactose and rhamnose coelute from this column (Slavin and Marlett, 1983). The amounts of sugars present were computed by a reporting integrator (Hewlett Packard model 3390A, Avondale, PA) after calibration data were obtained with vacuum-dried, standard sugars (Sigma). Erythritol (Sigma), added just before neutralization, was used as the internal standard. The component monosaccharides have been expressed as polysaccharides (X 0.9) following correction for residual starch, if present, and for hydrolysis losses which have been previously reported (Neilson and Marlett, 1983; Slavin and Marlett, 1983).

As previously described (Neilson and Marlett, 1983) uronic acids were determined colorimetrically by adapting the 3-hydroxydiphenyl (Pfaltz and Bauer, Inc., Stanford, CT) method of Blumenkrantz and Asboe-Hansen (1973) with D-galacturonic acid (Sigma) as the standard. Fiber residues were treated with strong acid, 72% sulfuric, for 3 hr at ambient temperature), diluted and vacuum filtered through glass fiber paper (Whatman, GFA). Uronic acids were expressed as a polysaccharide.

RESULTS

THE TOTAL DIETARY FIBER recovered from a food as NDF was generally similar to the total fiber recovered as the insoluble fraction by the Southgate method (Table 1). Lima bean NDF was less than the Southgate insoluble fraction, 13.6 vs 17.6% of the sample dry weight, but the NDF method recovered more fiber from shredded wheat than did the Southgate procedure, 9.1 vs 7.8%. The two methods consistently recovered different amounts of apparent lignin and neutral sugars although the differences were not always large. The NDF residue from each food contained more neutral sugars and less lignin than did the comparable insoluble fiber fraction. Uronic acids represented 4 to 6% of the total NDF or insoluble fiber except for the Southgate apple residue which was 8% uronic acids (Table 1).

The neutral saccharide compositions of the two fiber residues were similar for four of the five foods studied (Table 2). The NDF residue from oatmeal contained more xylose and arabinose and less mannose than did the Southgate-derived insoluble fraction. The NDF method also recovered relatively more xylose from the two wheat products, wheat bran and shredded wheat.

Recovery of the NDF and the extractive free residue obtained by the Southgate method ranged from 93.1 – 106.6% (Table 3). Major differences were detected in the crude protein content (N X 6.25) of the two fiber reisdues from all foods. The lignin content of the residues from lima beans and apples were also different. The larger amounts of crude protein and lignin in the Southgate extractive-free residue resulted in a relatively lower yield of neutral sugars (Table 3). The analytical procedure was reproducible (Table 4). When four separate lima bean NDF residues were subjected to quantitative analysis, over 90% of the residue was accounted for in each instance. The coefficients of variation exceeded 10% only when relatively small amounts of neutral sugars were measured by HPLC.

Starch was most effectively removed from fiber residues by the porcine amylase treatment of NDF recommended by Schaller (1976) (Table 5). There were no differences in the filtration rates of samples treated with the various enzymatic modifications. Measurement of starch and nitrogen in the residues was not attempted when the yield of fiber was very low. With the exception of puffed rice, only small

Table 1—Comparison of the compositions of insoluble dietary fiber fractions and NDF residues

	Neutral sugars	Lignin	Uronic acids	Dietary fiber
	------------- % of original dry wt.-------------			
WHEAT BRAN				
NDF	28.4 ± 0.6[a]	6.7 ± 0.4	1.5 ± 0.1	36.6 ± 0.2
Southgate	26.7 ± 2.8	8.3 ± 0.8	1.7 ± 0.1	36.7 ± 1.9
LIMA BEANS				
NDF	13.0 ± 0.2	0.0	0.6 ± 0.0	13.6 ± 0.2
Southgate	10.3 ± 0.2	6.3 ± 0.7	1.0 ± 0.0	17.6 ± 0.2
OATMEAL				
NDF	3.6 ± 0.5	0.9 ± 0.3	0.2 ± 0.0	4.6 ± 0.2
Southgate	2.0 ± 0.2	2.8 ± 0.0	0.1 ± 0.0	4.9 ± 0.3
APPLES				
NDF	6.9 ± 0.3	tr	0.4 ± 0.0	7.3 ± 0.3
Southgate	3.5 ± 0.2	3.3 ± 0.0	0.6 ± 0.0	7.4 ± 0.2
SHREDDED WHEAT				
NDF	7.4 ± 0.0	1.4 ± 0.4	0.4 ± 0.0	9.1 ± 0.3
Southgate	5.5 ± 0.7	2.1 ± 0.1	0.3 ± 0.0	7.8 ± 0.6

[a] Mean ± SD, n = 2.

amounts of starch remained in the NDF residues of grain products when either amylase pretreatment or midtreatment was used. Significant amounts of starch remained in the legume NDF residues subjected to the amylase pre- or midtreatment and neither the Schaller modification nor the Southgate approach removed starch from peas (Table 5). The Southgate approach to starch removal was not as effective and 5 – 10% of the residue weights of 5 of the 11 foods studied was starch (Table 5). As expected, the neutral detergent solution, compared to the Southgate extraction, solubilized nitrogen from the fiber residues. Gravimetric quantitation of NDF or of the Southgate-derived insoluble fiber fraction usually yielded a fiber content similar to the chemically determined value, although each of the Southgate residues had to be corrected for starch and/or crude protein contents (Table 6).

DISCUSSION

THE DATA from this study in which the compositions of NDF and the Southgate-derived insoluble fiber fraction were compared, combined with previous comparisons of

NDF composition and the insoluble fiber fraction obtained by the method of Theander (Neilson and Marlett, 1983), strongly suggest that NDF represents the insoluble fraction of dietary fiber. In the present study the largest compositional difference between the two methods was in the yield of apparent lignin. This is also the least sensitive step of the analytical procedure since it is gravimetric. It is theoretically possible that the relatively larger yield of lignin represented incomplete hydrolysis of the Southgate residues. However, earlier work from this laboratory, in which wheat bran apparent lignin was rehydrolyzed, indicates that hydrolysis of the sugars in the insoluble residues was complete (Neilson and Marlett, 1983). The Southgate residues did contain significant amounts of crude protein. Thus, a more plausible hypothesis is that the apparent lignin fraction may include acid-resistant complexes of carbohydrate and nitrogenous components which were formed during the extraction steps prior to acid hydrolysis. This hypothesis would be consistent with our observation that more apparent lignin and less neutral sugars were recovered by the Southgate residue when compared to the NDF residue (Table 1). The relatively low yield of apparent

Table 2—Neutral saccharide compositions of insoluble fiber fractions and NDF residues

	Cellobiose	Glucose	Xylose	Gal/Rham	Arabinose	Mannose
			% of total			
WHEAT BRAN						
NDF	3.9 ± 0.1[a]	26.2 ± 0.3	44.2 ± 0.1	0.0	25.7 ± 0.0	tr
Southgate	3.1 ± 0.3	26.4 ± 2.2	34.9 ± 2.4	5.1 ± 2.0	30.5 ± 2.9	0.0
LIMA BEANS						
NDF	4.4 ± 0.7	69.8 ± 0.1	16.5 ± 0.0	0.0	9.2 ± 0.6	0.0
Southgate	8.9 ± 0.3	61.1 ± 3.0	15.3 ± 0.0	0.0	6.7 ± 1.3	8.0 ± 4.2
OATMEAL						
NDF	1.8 ± 0.2	35.7 ± 4.1	34.0 ± 2.1	2.9 ± 0.0	22.1 ± 0.8	3.6 ± 1.1
Southgate	3.6 ± 0.0	36.3 ± 1.3	22.4 ± 0.3	3.3 ± 0.0	10.2 ± 0.5	24.2 ± 1.5
APPLES						
NDF	7.0 ± 1.1	51.9 ± 1.6	12.9 ± 0.1	8.1 ± 0.0	14.9 ± 0.1	5.5 ± 0.4
Southgate	6.8 ± 0.1	45.7 ± 3.0	14.8 ± 0.8	9.4 ± 0.6	14.2 ± 1.2	8.8 ± 0.7
SHREDDED WHEAT						
NDF	1.6 ± 0.0	30.0 ± 0.7	44.2 ± 4.4	2.1 ± 0.3	21.2 ± 5.2	1.0 ± 0.2
Southgate	5.6 ± 1.5	30.5 ± 1.7	32.1 ± 1.3	3.1 ± 0.7	21.0 ± 0.6	7.8 ± 2.5

[a] Mean ± SD, n = 2.

Table 3—Recovery of extractive free residue during quantitative dietary fiber analysis

Sample	Neutral sugars[a]	Starch	Protein[b]	Lignin	Uronic acids	Recovery
			% of extractive free residue			
WHEAT BRAN						
NDF	69.7 ± 0.2[c]	0.0	4.7 ± 0.0	16.5 ± 0.4	3.7 ± 0.1	94.7 ± 0.2
Southgate	58.0 ± 6.2	0.0	17.0 ± 1.7	18.1 ± 1.8	3.8 ± 0.2	96.9 ± 2.5
LIMA BEANS						
NDF	95.2 ± 3.2	0.0	0.0	0.0	4.0 ± 0.0	99.2 ± 3.2
Southgate	34.9 ± 1.9	2.7 ± 0.0	43.7 ± 0.0	21.7 ± 2.1	3.6 ± 0.0	106.6 ± 0.2
OATMEAL						
NDF	72.0 ± 11.7	0.0	3.5 ± 0.5	18.9 ± 8.6	3.8 ± 0.0	98.4 ± 2.6
Southgate	14.6 ± 1.8	3.0 ± 0.0	53.7 ± 0.0	20.9 ± 0.1	0.9 ± 0.0	93.1 ± 2.0
APPLES						
NDF	96.4 ± 0.0	0.0	0.0	0.0	5.5 ± 0.0	102.0 ± 5.0
Southgate	39.9 ± 2.5	0.0	12.9 ± 1.1	36.8 ± 0.4	6.5 ± 0.0	96.0 ± 1.0
SHREDDED WHEAT						
NDF	77.0 ± 3.2	0.5 ± 0.0	0.8 ± 0.0	14.0 ± 3.6	3.9 ± 0.2	96.2 ± 0.2
Southgate	44.3 ± 5.6	5.1 ± 0.0	27.1 ± 0.0	17.2 ± 0.6	2.1 ± 0.0	95.7 ± 5.0

[a] Determined by HPLC.
[b] N x 6.25.
[c] Mean ± SD, n = 2.

lignin in the NDF may be due in part to the presence of sodium sulfite in the detergent solution which has been reported to solubilize some lignin (Robertson and Van Soest, 1981). However, we were unable to demonstrate any effect of sodium sulfite on the yield of apparent lignin from peas. Negligible lignin was recovered in the absence as well as in the presence of sodium sulfite (Chesters and Marlett, unpublished), whereas about 1/6 of the quantitatively analyzed insoluble fiber from peas obtained by the Theander method was apparent lignin (Neilson and Marlett,

1983). Fiber residues extracted by this method also contain significant amounts of crude protein (Neilson and Marlett, 1983).

Generally, the NDF and the Southgate insoluble fiber residues contained similar and only small amounts of uronic acids, even though the foods which were analyzed were very different. A larger uronic acid fraction than that in either the NDF or Southgate residue was recovered from apple by the Theander method of extracting insoluble fiber components (Neilson and Marlett, 1983).

Table 4—Variation in extraction and quantitative analysis of NDF from lima beans

Sample	Cellobiose	Glucose	Xylose	Gal/Rham	Arabinose	Mannose	Neutral sugars	Uronic acids	Recovery	NDF[b]
	--------------------------- % of Total Neutral Sugars ---------------------------						------- % of Ext-Free Residue[a] ---------------------			
1	4.9	69.8	16.5	0.0	8.8	0.0	93.0	4.0	97.0	13.4
2	4.0	69.9	16.5	0.0	9.6	0.0	97.4	4.0	101.4	13.7
3	1.3	75.8	14.1	1.6	7.3	0.0	88.4	4.0	92.4	13.1
4	1.6	72.4	14.6	1.7	9.7	0.0	98.9	4.0	102.9	15.5
Mean ± SD	3.0 ± 1.8	72.0 ± 2.8	15.4 ± 1.3	0.8 ± 1.1	8.9 ± 1.1		94.4 ± 4.7	4.0 ± 0.0	98.4 ± 4.7	13.9 ± 1.1
C.O.V.[c]	60.0	3.9	8.2	115.2	12.5		5.0	0.0	4.8	7.8

[a] Did not contain measurable amounts of starch, nitrogen or apparent lignin.
[b] Sum of fiber components, neutral sugars and uronic acids, expressed as % of original dry weight.
[c] Coefficient of variation

Table 5—Starch and nitrogen in NDF and water-insoluble fiber residues from human foodstuffs[a]

	--- Neutral Detergent Fiber ---				Southgate water-insoluble fraction
	Unmodified	Amylase pretreatment	Amylase midtreatment	Amylase posttreatment	
Wheat Bran	45.2 ± 0.7[b]	40.7 ± 0.3	36.4 ± 0.4	40.8 ± 0.9	46.5 ± 0.6
Starch	0.8 ± 0.1[c]	1.2 ± 0.2	0.0	0.0	0.0
Nitrogen	1.8 ± 0.0[c]	1.5 ± 0.1	1.3 ± 0.1	0.7 ± 0.2	2.7 ± 0.3
Coarse Wheat Bread	8.6 ± 0.2	6.3 ± 0.0	5.6 ± 0.2	4.6 ± 0.2	15.2 ± 0.8
Starch	17.6 ± 1.4*	2.2 ± 0.4*	3.7 ± 0.3*	0.0	3.7 ± 1.0
Nitrogen	2.1 ± 0.1	2.2 ± 0.2*	2.3 ± 0.0*	0.7 ± 0.1	7.5 ± 0.5
Shredded Wheat	19.7 ± 0.8	10.7 ± 0.3	10.0 ± 0.1	10.4 ± 0.2	11.0 ± 0.3
Starch	26.8 ± 0.5*	3.3 ± 0.4	2.0 ± 0.3	0.6 ± 0.2	5.1 ± 0.4*
Nitrogen	1.2 ± 0.0*	—[d]	—	0.0	4.4 ± 0.1
Triscuits	20.0 ± 0.1	17.6 ± 0.4	18.3 ± 0.3	12.4 ± 1.8	13.6 ± 2.0
Starch	5.5 ± 0.7	0.0	0.0	0.0	10.4 ± 1.5
Nitrogen	0.5 ± 0.0*	—	—	0.0	4.0 ± 0.3
Oatmeal	6.7 ± 0.4	3.9 ± 0.1	4.1 ± 0.1	4.8 ± 0.2	6.9 ± 0.6
Starch	7.3 ± 1.0*	0.0	0.0	0.0	6.9 ± 0.1*
Nitrogen	2.2 ± 0.5*	1.4 ± 0.0*	1.7 ± 0.1*	0.0	9.1 ± 0.5
Rice Krispies	14.5 ± 0.9	3.4 ± 0.2	1.3 ± 0.1	0.8 ± 0.1	8.8 ± 0.2
Starch	72.1 ± 5.8	1.5 ± 0.1*	—	—	0.0
Nitrogen	4.0 ± 0.3	1.1 ± 0.2	—	—	13.9 ± 0.9
Macaroni	2.7 ± 0.1	2.0 ± 0.1	1.5 ± 0.0*	1.9 ± 0.0	7.3 ± 0.7
Starch	27.1 ± 4.6*	—	—	0.0	4.9 ± 1.1
Nitrogen	0.0	—	—	—	8.3 ± 0.4
Puffed Rice	11.3 ± 0.9	1.3 ± 0.1	0.5 ± 0.0*	0.7 ± 0.1	7.0 ± 0.8
Starch	82.9 ± 4.1*	10.6 ± 2.7	—	—	0.0
Nitrogen	1.3 ± 0.1	—	—	—	15.1 ± 0.4
Lima Beans, canned	55.2 ± 0.4	17.9 ± 1.4	14.6 ± 0.0	15.2 ± 0.1	29.3 ± 0.7
Starch	94.1 ± 0.7*	20.9 ± 1.1*	6.2 ± 1.3	0.0	2.8 ± 0.3
Nitrogen	0.0	—	—	0.0	7.0 ± 0.8
Peas, canned	26.3 ± 1.6	16.6 ± 0.5	17.1 ± 0.3	18.2 ± 0.6	27.5 ± 0.2
Starch	30.7 ± 2.6	—	12.8 ± 1.2	6.5 ± 0.9	8.7 ± 1.1
Nitrogen	0.0	0.0	0.0	0.0	4.5 ± 0.1
Apple Pulp	9.5 ± 0.2	8.6 ± 0.2	9.8 ± 0.7	8.8 ± 0.5	8.6 ± 0.2
Starch	0.0	—	—	0.0	0.0
Nitrogen	0.0	—	—	0.0	2.1 ± 0.1

[a] n = 3 except * where n = 2.
[b] % of original sample dry weight.
[c] % of fiber residue.
[d] Not determined.

Table 6—Comparison of dietary fiber values determined by analysis vs. gravimetrically

Sample	NDF		Insoluble Residue	
	Analytical	Gravimetric	Analytical	Gravimetric
	% dry weight			
Wheat Bran	36.6	39.2[a]	36.6	39.3[a]
Lima Beans	13.6	15.2	17.6	16.8[a]
Oatmeal	4.6	4.8	4.9	2.8[a]
Apple	7.3	7.3	—[b]	—
Shredded Wheat	9.1	10.3[a]	7.8	7.7[a]

[a] Corrected for starch and/or crude protein (N x 5.7) content.
[b] Not determined.

Quantitative analysis of the constituent neutral sugars does provide some information about the major polysaccharides in a particular food fiber. The neutral sugar composition of the foods determined in this study indicate that the insoluble fiber fraction contains, in some instances, significant amounts of hemicellulose as well as cellulose. The major polysaccharide in wheat bran and shredded wheat was an arabinoxylan which accounted for about 55 to 65% of the neutral sugars. These data are consistent with the work of Brillouet et al. (1982) in which the major polysaccharide in wheat bran was a complex heteroxylan composed primarily of equivalent amounts of xylose and arabinose. The wheat products however did contain 30 – 35% glucans, as indicated by the combined amounts of cellobiose and glucose. In contrast to the relatively large hemicellulose component in wheat products, glucans were major components of lima beans and apples and probably reflect cellulose. Oatmeal polysaccharide composition was complex and differed with the method of analysis. Extraction of oatmeal with neutral detergent solution recovered a major arabinoxylan fraction, about 55% of the total neutral sugars, whereas the Southgate procedure extracted a residue composed of about 1/3 glucans, 1/3 arabinoxylans and 1/4 mannans. Some of the differences in the relative distribution of sugar between xylose and galactose/rhamnose, and between arabinose and mannose conceivably could reflect the fact that these two pairs of sugars are not completely resolved by the HPLC column (Neilson and Marlett, 1983). However, any differences attributable to the partial resolution are likely to be small, particularly since the variability between the analysis of duplicate samples was small.

It is generally agreed that dietary fiber in human foods consists of a labile fraction, extracted with hot water, and a stable or insoluble fraction which is recovered as a residue following this extraction step. The physiological effects of these two components differ, as well. Available evidence indicates that the labile components affect primarily small bowel function, whereas the stable components modulate large bowel function. Although relatively few foods have been quantitatively analyzed, it appears that the insoluble fraction is the major component of total dietary fiber.

The soluble and insoluble fractions of fiber from 11 foods have been individually quantitated and in all instances, the insoluble fraction accounted for ≥75% of the total dietary fiber in the food (Theander and Aman, 1979a, b; Theander and Aman, 1981; Neilson and Marlett, 1983). Only rye biscuit and flour, potato, apple and a food composite representing a day's intake of a typical low fiber diet had ≥15% soluble fiber components. Less than 15% of the total dietary fiber from lettuce, cabbage, carrot, wheat and rye brans and peas consisted of water soluble fiber components. A larger soluble fiber fraction has been reported only when those procedures with an acid step are used which solubilizes some of the labile fiber carbohydrates (Rasper, 1981; Schweizer and Wursch, 1981, Johans-

son et al., 1982). For example, 20 – 75% of the fiber arabinose was lost when food samples were incubated with pepsin, pH 1 for 18 hr at 37°C, as is done in the Johansson et al. (1982) modification of the Hellendorn et al. (1975) method. The variability in the amount of fiber solubilized was dependent on the food analyzed and is a significant negative feature of this method. We have found that quantitative analysis of the insoluble residue is technically easier than recovering and measuring the relatively small amounts of fiber carbohydrates from a dilute solution of fiber components, e.g. the water-soluble fraction. Thus, the better analytical approach would be to utilize a method which consistently recovers the major fraction of the total dietary fiber in a form more amenable to quantitative analysis.

Gravimetric estimation of the insoluble fiber fraction using the NDF method would be a simple convenient way to measure not only the insoluble components of dietary fiber but also the major fraction of the total. However, the NDF method must include a step to remove starch from the residue. In the present study, the gravimetrically determined NDF was within 10% of the chemically determined fiber value for all foods analyzed except shredded wheat where the difference was 12% when corrections for starch and crude protein were applied to the gravimetric value. Most of these differences would fall within the range of experimental error when fiber content was expressed on a fresh weight basis. The abilities of the neutral detergent solution to solubilize protein and of the amylase step to remove starch from most residues are clearly advantages of this method. The inability to remove starch completely from peas has been reported when other methods of starch extraction have been tested (Neilson and Marlett, 1983). Incomplete starch removal from kidney bean NDF also has been reported (Marlett and Lee, 1980). Together, the findings suggest that starch in legumes is difficult to remove enzymatically without prior gelatinization under pressure, similar to that which is used in the procedure to measure starch. Such a gelatinization step in a fiber analysis scheme before extraction of the water soluble fiber fraction would invariably result in the loss of some of the soluble fiber components. To obtain an accurate value for the glucose content of the fiber acid hydrolysate, it is essential to correct for starch contamination.

REFERENCES

Anderson, N.E. and Clydesdale, F.M. 1980. An analysis of the dietary fiber content of a standard wheat bran. J. Food Sci. 45: 336.

Asp, N. and Johansson, C. 1981. Techniques for measuring dietary fiber: Principal aims of methods and a comparison of results obtained by different techniques. Ch. 11. In "The Analysis of Dietary Fiber in Food." (Eds.), W.P.T. James and O. Theander, p. 173. Marcel Dekker, Inc., New York, NY.

AOAC. 1980. "Official Methods of Analysis," 13th ed., p. 222. Association of Official Analytical Chemists, Washington, DC.

Blake, J.D. and Richards, G.N. 1970. A critical re-examination of problems inherent in compositional analysis of hemicelluloses by gas-liquid chromatography. Carbohyd. Res. 14: 375.

Blumenkrantz, N. and Asboe-Hansen, G. 1973. New method for quantitative determination of uronic acids. Anal. Chem. 54: 484.

Brauer, P.M., Slavin, J.L., and Marlett, J.A. 1981. Apparent digestibility of neutral detergent fiber in elderly and young adults. Am. J. Clin. Nutr. 34: 1061.

Brillouet, J., Joseleau, J., Utille, J., and Lelievre, D. 1982. Isolation, purification and characterization of a complex heteroxylan from industrial wheat bran. J. Agric. Food Chem. 30: 488.

Englyst, H. 1981. Determination of carbohydrate and its composition in plant materials. Ch. 6. In "The Analysis of Dietary Fiber in Food," (Eds.) W.P.T. James and O. Theander, p. 71. Marcel Dekker, Inc., New York, NY.

Furda, I. 1981. Simultaneous analysis of soluble and insoluble dietary fiber. Ch. 10. In "The Analysis of Dietary Fiber in Food," (Eds.) W.P.T. James and O. Theander, p. 163. Marcel Dekker, Inc., New York, NY.

Goering, H.K. and Van Soest, P.J. 1970. "Forage Fiber Analysis, Handbook 379," p. 1. USDA, Washington, DC.

Hellendoorn, E.W., Nordhoff, M.G., and Slagman, J. 1975. Enzymatic determination of neutral the indigestible residue (dietary fibre) content of human food. J. Sci. Food Agric. 26: 1461.

Effects of Microwave Heating on Solubility, Digestibility and Metabolism of Soy Protein

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────────────────ABSTRACT────────────────

Microwave heating of soybeans for 9 min decreased protein solubility from 80 to 17%, from 81 to 18%, and from 72 to 16% when deionized H_2O, 0.6N NaCl and 0.4N $CaCl_2$ were used as solvents, respectively. Experiments were conducted to determine *in vivo* protein digestibility and metabolizable nitrogen using male Sprague-Dawley rats. The percentages of true digestibility were found to be 73, 84, 87 and 81 when the soybeans were microwave heated for 0, 9, 12 and 15 min, respectively. Microwave heating soybeans up to 15 min did not alter the fatty acids composition of the beans.

INTRODUCTION

VARIOUS HEATING METHODS have been used to improve the nutritional value of soybeans, partially by investigating trypsin inhibitor and lipoxygenase activities (White et al., 1967; Pour-El and Peck, 1973; Pour-El et al., 1981; Ahrar and Schinogethe, 1979; Hill and Harshburger, 1979; Collins and Beaty, 1980; McNaughton and Reece, 1980; Hafez et al., 1984). The ingestion of diets containing raw soybeans by chicks caused a marked reduction in the total proteolytic activity in the small intestine (Alumot and Nitsan, 1961; Gertler et al., 1967). No such effect was observed with rats (Nitsan and Alumot, 1965). Rats fed diets containing raw soybean meal showed higher intestinal proteolytic activity (Haines and Lyman, 1961; Kayamabashi and Lymar, 1966).

This study was conducted to evaluate the effects of microwave heating on the solubility and digestibility of soybean protein, and its effect on intestinal proteolytic activity in rats. The effect of microwave heating on the fatty acid composition of the soy oil was also studied.

MATERIALS & METHODS

COMMERCIAL SOYBEANS from Essex Cultivar were used. The beans were cleaned and divided for experimental treatment. They were tested for moisture, 7.5% (AOAC, 1980) and held in zip bags at 4°C until heated.

Microwave heating

One kilogram batches of whole soybeans were heated in the microwave oven for 0.0, 9.0, 12.0 and 15.0 min at temperatures determined as reported earlier (Hafez et al., 1984). A modified domestic size Kenmore microwave oven (Model 99872, Sears Roebuck, Co.) was used. The oven was rated to 650 watts (full power) at 2450 MHz and was operated at full power. To obtain uniform heating, a turntable was installed which slowly rotated the sample in a direction opposite to the built-in microwave stirrer. The sample was placed in a cylindrical shell container of 2.54 cm thickness.

Nitrogen solubility

Microwave treated samples (100g) were ground in a Wiley Mill, passed through a 60-mesh screen and divided into two portions.

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The oil was extracted with hexane, and the defatted soybean samples were used to determine the solubility of soy protein before and after treatment. Nitrogen solubility was determined by extraction with H_2O, NaCl or $CaCl_2$ for 2 hr at room temperature (23°C). Different concentrations of solvents were used to determine the conditions of highest nitrogen solubility. The concentration ranges of the solvents were 0.1 – 1.0N for NaCl and 0.1 – 0.6N for $CaCl_2$. The pH ranged from 4.5 – 8.5. The optimum conditions for the extraction of nitrogen were 0.6N for NaCl and 0.4N for $CaCl_2$ at pH 6.6 – 7. The insoluble and soluble fractions were separated by centrifugation at 10,000 x g for 15 min. Total and soluble nitrogen were determined by the micro-Kjeldahl procedure (AOAC, 1980).

Determination of fatty acids

The fatty acid methyl esters (FAME) were prepared from the lipid extract as follows: 5.0 mL of chloroform:methanol (2:1) (Folch et al., 1957) lipid extract of soybean samples were evaporated to dryness with stream of nitrogen, and 2 mL each of methanol and solution of 80% KOH (W/V) was added. The contents were shaken in a 40°C water bath for 20 min, cooled to room temperaure (23°C) and shaken vigorously with 20 mL of resdistilled hexane. After standing for 15 min, the aqueous layer was transferred into a 50 mL conical flask, and two drops of methyl orange was added, followed by acidification with HCl (1:1 v/v). The liberated fatty acids were extracted three times with hexane using 10 ml portion for each extraction. The combined fatty acid extract was dried over anhydrous sodium sulfate and the solvent was evaporated with stream of nitrogen. The fatty acids were dissolved in diethyl ether and methanol (2:1), and methylated by the dropwise addition of diazomethane solution until the yellow color persisted and the bubbles of nitrogen gas ceased (Osman et al., 1978). The mixture was dried with a stream of nitrogen. Finally, the methylated fatty acids were dissolved in hexane and an aliquot (2 mL) of the solution was analyzed by gas liquid chromatography. A Varian model 2400 GLC equipped with flame ionization detector and coiled stainless steel column (2m x 4mm) packed with 10% Degs supported on acid washed diatomite G (100/120 mesh) and connected with Model CDS-111 chromatography data systems (Varian, Palo Alto, CA) was used. Fatty acid standards were purchased from Sigma Company (St. Louis, MO) and methylated as mentioned above.

In vivo digestibility

Two kilograms of microwave heated soybeans were ground to 0.64 cm mesh in a hammer mill and defatted by using hexane in a 2.2L capacity fat soxhlet extractor; solvent was evaporated with a stream of air at room temperature. Diets were made with the soybean meal as the sole source of protein. The composition of the diet is shown in Table 1. Male Sprague-Dawley rats, having an initial average weight of 90g were divided into five groups of two rats. The first group was fed the protein-free diet; the second group received a diet containing raw soybean; the third, fourth and fifth groups were fed diets containing soybean microwave heated at different times. Rats were fed *ad. libitum* and body weights were determined daily. The feces were collected daily from each cage, dried at 95°C for 5 hr, weighed, ground and stored in the freezer until analyzed for nitrogen. Dried feces (0.5g) was used to determine fecal nitrogen by the micro-Kjeldahl method (AOAC, 1980).

Calculation of protein digestibility of soybean

$$\frac{\text{Dietary N} - (\text{Fecal N} - \text{Endogenous Fecal N})}{\text{Dietary N}} \times 100$$

Calculation of metabolizable nitrogen of soybean

$$\frac{\text{Dietary N-(Fecal N} - \text{Endogenous Fecal N} - \text{Urinary N} - \text{Endogenous Urinary N)}}{\text{Dietary N}} \times 100$$

Endogenous fecal and urinary nitrogen are from endogenous source (body tissues) but are not from exogenous source (food). Endogenous fecal and urinary nitrogen are equivalent to the fecal and urinary nitrogen excreted by rats fed a nitrogen-free diet (Calloway and Margen, 1971).

The experimental diets were introduced to the animals for 7 days. The animals were sacrificed at the end of the experimental period. Four inches of the small intestine from the posterior end of the duodenum was immediately removed. The chyme was washed off, and the intestine was sliced and homogenized in 4 mL of saline solution. The homogenate was centrifuged for 60 min (40,000 x g) at 2°C. The supernatant was used to determine proteolytic enzyme activities using the method of Kakade et al. (1969).

RESULTS & DISCUSSIONS

THE EFFECTS of microwave heating of whole soybeans on nitrogen solubility are shown in Table 2. The total nitrogen of soybean meal (8.22%) was not affected by microwave heating. For a raw soybean meal, the solubility in deionized H_2O and NaCl was similar (80.3% and 81.1%, respectively), and higher than the solubility in $CaCl_2$ (72.3%). Microwave heating for 9 and 12 min drastically reduced the solubility in deionized H_2O (17.5% and 17.3%); in NaCl (18.4% and 15.4%); and in $CaCl_2$ (15.7% and 14.4%). There was a slight decrease in solubility in the case of 15 min microwave heating (15.5%, 12.7% and 12.0% for deionized H_2O, NaCl and $CaCl_2$, respectively).

Earlier reports (Clark et al., 1977; Berrig et al., 1974; Schingoethe and Ahrar, 1977) indicated that heat treatment, either dry or moist, caused a considerable decrease in solubility of soybean meal protein. As the soybean meal was heated, the relative proportion of the alubmin and

globulin fraction decreased accompanied by a corresponding reduction in nitrogen solubility (Clark et al., 1977). These studies also demonstrated that heating caused irreversible disruption of the quarternary structure of globulin, the major component of soybean proteins (Mori et al., 1981; Yamagishi et al., 1980).

The present study indicated that microwave heating had the same effect even though it is electromagnetic in character. The extraction of soyprotein using NaCl and/or $CaCl_2$ did not improve the protein solubility of microwave heated soybeans. This may be due to the irreversible disruption of the quarternary protein structure.

The fatty acid composition of raw and microwave treated soybean is given in Table 3. There was no quantitative or qualitative differences in the composition between raw and microwave treated soybean. Mai et al. (1980) found that the fatty acid composition of peanuts remained unchanged after microwave cooking up to 15 min. Our data are in agreement with the data of Mai et al. (1980). These data indicated that microwave heating did not change the fatty acid pattern of the soybeans. Lipoxygenase activity was greatly decreased by microwave heating (Pour-El and Peck, 1973; Hafez et al., 1983). The data of fatty acids analysis showed that soybean oil appeared nutritionally superior to other bean seeds oil (winged beans. Hafez et al., 1984; peanuts, Mai et al., 1980) due to higher unsaturated fatty acid content. Based on our report (Hafez et al., 1983) and available information, we can suggest that microwave heating of whole soybeans before grinding to extract the oil, will protect unsaturated fatty acids from being oxidized by the enzyme lipoxygenase and hence will improve the nutritional quality of soybean oil.

Table 4 illustrates the *in vivo* protein digestibility for raw and microwaved heated soybean for male Sprague-Dawley rats. The digestibility increased for microwave heating periods of 9 and 12 min (84.23% and 87.00%, respectively), and then declined at 15 min of microwave treatment. Decrease in the solubility of soy protein with increasing microwaving time was accompanied with increase in digestibility. These results agree with the fact that proper heat treatment increases the digestibility of legume proteins (Rhee and Rhee, 1981, Hsu et al., 1977). Soyflour or soy grits with

Table 1 — Diet composition for in vivo digestibility

Ingredients	Soybean diet (grams)	Protein free diet (grams)
Defatted Soybean Meal[a]	400	0
Corn Oil	50	50
DL-Methionine	4.5	0
AIN Mineral Mix[b]	40	40
AIN Vitamin Mix[b]	20	20
Dextrose	210	420
Corn Starch	225.5	420
Alpa-Cell[b]	50	50

[a] The microwaving time varied with each of four diet, 0, 9, 12, and 15 min; whole soybeans were microwaved in 1 kg batches, then the beans were ground and defatted using hexane.
[b] Mineral mixture and vitamin fortification mixture and alpha cells purchased from ICN nutritional biochemicals (Cleveland, OH).

Table 3 — Effect of microwave heating on the fatty acid composition of soybean oil

Fatty acids		Microwave heating time, min			
		0	9	12	15
Palmitic	$C_{16:0}$	10.71	10.23	10.91	10.27
Stearic	$C_{18:0}$	3.55	3.61	3.65	3.38
Oleic	$C_{18:1}$	19.43	19.35	19.55	20.24
Linoleic	$C_{18:2}$	57.64	57.81	57.81	57.81
Linolenic	$C_{18:3}$	8.57	8.81	8.03	8.30

Table 2 — Effect of microwave heating on the nitrogen solubility of soybean

Microwaving time, min.	Total[b] nitrogen %	Solvents[a]					
		Deionized H_2O		0.6N NaCl		0.4N $CaCl_2$	
		Soluble nitrogen %	Solubility %	Soluble nitrogen %	Solubility %	Soluble nitrogen %	Solubility %
0	8.22	6.60	80.3	6.67	81.1	5.94	72.3
9	8.22	1.44	17.5	1.51	18.4	1.29	15.7
12	8.22	1.42	17.3	1.23	15.0	1.18	14.4
15	8.22	1.27	15.5	1.04	12.7	0.99	12.0

[a] The ratio of soybean meal: solvent was 1:20 (W/V).
[b] Total nitrogen (soluble and insoluble) contents of the defatted soybean meal per 100g dry weight.

Table 4—Effect of microwave heating on the in vivo digestiblility of soybean protein, metabolizable nitrogen and intestinal proteolytic activity[a]

Treatment	Body weight gain 7 days (grams)	Protein digestibility (%)	Metabolizable nitrogen (%)	Intestinal proteolytic activity, units/mg Protein[b]
Nitrogen free diet[c]	−17.6 ± 0.40	−	−	15.0 ± 2.0
Microwaving Time, min				
0	28.0 ± 5.82	72.969 ± 2.046	68.938 ± 2.242	21.0 ± 0.20
9	70.6 ± 4.92	84.251 ± 0.472	79.764 ± 0.242	23.1 ± 2.3
12	73.8 ± 3.62	87.056 ± 0.853	83.207 ± 0.918	26.5 ± 3.2
15	63.3 ± 3.00	80.728 ± 0.88	76.230 ± 8.71	39.7 ± 1.5

[a] Data are reported as Mean ± SEM
[b] One unit is defined as an increase of 0.01 absorbance units at 280 nm in 20 min per 10 mL of the reaction mixture under the condition defined herin.
[c] This group was used to determine the endogenous fecal and urinary nitrogen.

lower solubility index (PSI) and lower protein dispersion index (PDI) values contain less active trypsin inhibitor and more denatured protein which cause increases in protein digestibility in vitro (Hsu et al., 1977). The reduction in digestibility after 12 min of microwave treatment was probably due to the Maillard reaction and the formation of browning substances which were found after microwave heating. Rhee and Rhee (1981) reported decreases in the digestibility of protein in vitro when browning substances increased. Hafez and Mohamed (1983) also found that the browning substances have proteolytic inhibitor activity.

Data on the metabolizable nitrogen of raw and microwave treated soybeans are shown in Table 4. In this experiment higher digestibility and metabolizable nitrogen were observed when the soybeans were microwave heated for 12 min. The correlation coefficient of digestibility and metabolizable nitrogen was found to be 0.999. The linear regression equation was calculated to be $Y = 0.999 X −4.167$, where X is digestibility and Y is metabolizable nitrogen.

Rats fed the microwave heated soybean meal showed better growth than rats fed the raw soybean meal (Table 4). Higher body weight gain was obtained when soybeans were microwave heated for 9 and 12 min (70.6 and 73.8 g/body gain/7 days, respectively). On the other hand, lower body weight gain was obtained when the animals were fed soybean microwave heated for 15 min. This may be attributed to the increase of browning substances which was reported earlier by Hafez et al. (1984) when the soybean seeds were heated by using a microwave oven.

High correlation between body weight gain and metabolizable nitrogen ($r = 0.978$) was observed. This correlation was slightly higher than the correlation between the digestibility and body weight gain ($r = 0.958$). The linear regression equations are: $W = 3.908 X −234.992$ and $W = 3.914 Y −250.429$ where W, X and Y stand for body weight gain, metabolizable nitrogen and digestibility, respectively.

The effect of raw and microwave heated soybean diets on the intestinal proteolytic activity is shown in Table 4. The data indicated that the proteolytic activity increased with microwave heating time (23.1, 26.5 and 39.7 units/mg protein for 9, 12 and 15 min). Positive correlation ($r = 0.788$) was observed between microwave heating time and proteolytic activity. The highest enzymatic activity accompanied by a corresponding reduction in digestibility and metabolizable nitrogen (80.73 and 76.23%, respectively) was recorded for the 15 min microwave heated soybean.

Since the browning substances have proteolytic inhibitor activity as mentioned above, we suggested that the increase in proteolytic activity may be caused by hypersection of proteolytic enzyme by the pancrease due to the increase in

browning substances. Browning substances inhibited proteolytic enzymes in vitro (Rhee and Rhee 1981, Hsu et al., 1977), but their mechanism in vivo is still unknown. In case of raw soybean diet, trypsin may be complexed with the inhibitors introduced into the intestinal tract driving the equilibrium pancreas trypsinogen ⇌ trypsin to the right, thus depleting the trypsinogen and causing hypersecretion which in turn causes pancreatic hypertrophy. The microwave treatment stops the pancreatic hypertrophy by destroying the trypsin inhibitors (Hafez et al., 1983). Both inhibited proteolytic activity, and pancreatic hypertrophy appeared to be involved in the growth depression of the animal fed raw soybean diet.

In contrast, Nitsan and Bondi (1965) indicated that there were no differences in the effect of raw and heated soybeans on the level of proteolytic activity in the small intestine of rats. They indicated that their results were probably due to the nonspecific substrate (casein) which was used to measure proteolysis along the entire digestive tract. In our experiment, 10 cm of the small intestine from the posterior end of the duodenum was used to measure proteolysis.

It should be pointed out that proper microwave treatment increased the digestibility of soybean. Optimum microwave heating time was around 9 – 12 min for 1 kg whole soybeans to improve weight gain, digestibility and intestinal proteolytic activity under the experimental conditions in this study.

REFERENCES

Ahrar, M. and Schinogethe, D.J. 1979. Heated soybean as a protein supplement for lactating cows. J. Dairy Sci. 62: 932.
Alumot, E. and Nitsan, Z. 1961. The influence of soybean antitrypsin on the intestinal proteolysis of the chick. J. Nutr. 73: 71.
AOAC, 1980. "Official Methods of Analysis," 13th ed., Association of Official Analytical Chemists, Washington, DC.
Berrig, R.G., Clark, H.H., and Davis, C.L. 1974. Effect of abomasal infusion of sodium caseinate on milk yield, nitrogen utilization and amino acid nutrition of the dairy cow. J. Nutr. 104: 151.
Calloway, D.H. and Margen, S. 1971. Variation in endogenous nitrogen excretion and dietary nitrogen utilization as determinants of human requirements. J. Nutr. 101: 205.
Clark, J.H., Spires, H.R., Berrig, R.G., and Bennik, M.R. 1977. Milk production, nitrogen utilizaton and glucose synthesis in lactating cows infused postruminally with sodium caseinate and glucose. J. Nutr. 107: 631.
Collins, J.L. and Beaty, B.F. 1980. Heat inactivation of trypsin inhibitor in fresh green soybeans and physiological responses of rats fed the beans. J. Food Sci. 45: 542.
Folch, J., Lees, M., and Sloane-Starely, G.H.C. 1957. A simple method of isolation and purification of total lipids from animal tissues, J. Biol. Chem. 226: 497.
Gertler, A., Birk, Y., and Bondi, A. 1967. A comparative study of nutritional and physiological significance of pure soybean trypsin inhibitors and of ethanol-extracted soybean meals in chicks and rats. J. Nutr. 91: 358.
Hafez, Y.S. and Mohamed, A.I. 1983. Presence of nonprotein trypsin inhibitor in soy and winged beans. J. Food Sci. 48: 75.
Hafez, Y.S., and Mohamed, A.I., and Herath, W. 1984. Nutritive composition of different varieties of winged bean seeds and tuber. Nutr. Rept. Intern. 29(2): 253.

In Vivo and In Vitro Assessment of Antinutritional Factors in Peanut and Soy

H. S. SITREN, E. M. AHMED, and D.E. GEORGE

―――――――――――― ABSTRACT ――――――――――――

Raw and heat processed peanut flours were found to contain higher in vitro trypsin inhibitor activity and lectin content than similarly processed soy flour. When fed to weanling rats at a level of 10% protein, the response pattern in food consumption, growth, liver function, and serum chemistries was not consistent among or between the groups. It is concluded that the levels of antinutritional factors in legumes do not correlate with their overall biological impact in feeding studies with the rat.

INTRODUCTION

MUCH IS KNOWN about the depression in growth and altered pancreatic function of rats fed raw or dry-heat processed soy (Liener, 1981; Korgdahl and Holm, 1979; Rackis, 1974; Turner and Liener, 1975). These effects are believed to be mediated primarily by the action of certain antinutritional factors such as proteinase inhibitors and hemagglutinins or lectins. These factors are naturally present in soy but are largely inactivated by moist-heat processing (Liener, 1979).

Other legumes, such as peanut, also contain antinutritional factors but much less is known about the effects of heat processing on them and on growth and pancreatic function in rats fed this protein. Further, there is little or no information on the effects of feeding raw or processed legumes on other organs, such as the liver, and on the levels of blood chemistries such as serum proteins and lipids.

The purpose of this study was to: (1) quantitate the lectin content and trypsin inhibitory activity of soybean and peanuts; (2) determine the effects of processing methods on the activity of these antinutritional factors; and (3) characterize the effects on growth, liver function, and blood chemistries of rats fed raw or processed peanut and soy.

MATERIALS & METHODS

Preparation of flours

Soybeans of the Bragg variety were obtained commercially. They were ground in a Waring Blendor to a flour consistency. The product was then spread on a shallow pan and the hulls were blown free by an electric fan. The flour was collected and refrigerated. Partially defatted, deskinned peanuts of the Florunner variety were obtained commercially. The method of defatting was by low temperature pressing. The peanuts were ground and stored as described above.

Heat treatment was carried out as follows. A portion of each raw flour was placed in a shallow stainless steel pan at a thickness of about 1.0 cm, and then subjected to processing by one of two methods: dry heating in a forced air oven at 177°C for 20 min, or moist heating in an autoclave at 121°C and 15 p.s.i. for 20 min. After heating, the flours were allowed to cool at room temperature and refrigerated. Samples of the raw, dry-heated, and moist-heated flours, as well as food grade casein, were taken for the analysis of moisture content, Kjeldahl nitrogen (N) and crude lipid. These data

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were used in formulating the diets for the rat feeding studies.

In vitro studies

Two grams of flour were extracted with 100 mL of 0.85% sodium chloride for 2 hr at 2 – 3°C. The suspension was centrifuged at 15,000 rpm for 1 hr at 4°C. The supernatant was filtered through Whatman paper no. 3. The filtrate was then analyzed for TI activity and lectin concentration. TI activity was determined by a method modified from Kakade et al. (1974) and Krogdahl and Holm (1979) as reported by Ahmed et al. (1983). The reaction mixture contained: 2 mL of a 2 mg % solution of trypsin (Type III, Sigma Chemical), 5 mL of benzoyl-DL-arginine-p-nitroanilide (U.S. Biochemicals) dissolved in TRIS buffer, pH 9.55, prewarmed to 37°C, and 1 mL of the seed extract. The mixture was incubated at 37°C for exactly 10 min, inactivated with 1 mL of 30% acetic acid, and centrifuged for 15 min at 27,000 × g. The color intensity of the supernatant was measured at 410 nm. Sample blanks were included in each assay. A standard curve was prepared from purified soy TI (Type I-S, Sigma Chemical). Results are expressed as % inhibition of bovine trypsin on a synthetic peptide substrate. We have found that this procedure gives consistent and reproducible results. Casein, a product not known to contain TI, was assayed as a negative control and showed no TI activity.

Lectin content was determined on the same saline extracts by a slight modification of the photometric methods of Liener (1955), Herholzer and Suggs (1969), and Lotan et al. (1975). Type A human red blood cells (RBC) were obtained and pre-treated as follows: 50 mL of a 4% suspension of washed RBC were mixed with 10 units of neuramidinase (Sigma Chemical) and incubated at 37°C for 70 min. The treated RBC suspension was diluted to 1.5% RBC with 0.85% saline, pH 7.2 and then mixed with serial dilutions of the saline extracts from peanut and soy. Agglutination was allowed to proceed at room temperature. The most dilute concentration of extract which agglutinated the 1.5% RBC suspension after 150 min was used to calculate lectin concentration. The concentration is expressed as μg lectin in comparison with the agglutinating activity of purified, commercial peanut and soy lectins (No. L-0881 and Type I-S, respectively. Sigma Chemical). The minimum concentration of lectin which agglutinated the treated RBC was 0.062 μg/ml for peanut and 0.39 μg/ml for soy.

In vivo studies

Male, weanling Sprague-Dawley rats (Harlan industries, Indianapolis, IN) were housed individually in stainless steel cages with wire mesh bottoms. They were given a 10% casein control diet (Table 1) and water ad libitum for 1 wk. Groups of 10 rats were then assigned to receive one of seven experimental diets as outlined in Table 1. Food intake and body weight changes were measured regularly over the next 28 days. The amount of spilled food was measured and taken into account in the calculation of food intake of rats. Three to four rats from each group were then randomly selected for biochemial analyses. They were rapidly decapitated and trunk blood was collected in chilled tubes and allowed to clot. Serum was separated by centrifugation and frozen pending analysis for total protein (Yatzidis, 1977), albumin (Doumas et al., 1971), urea (Marsh et al., 1965), cholesterol (Zlatkis, 1953), and triglycerides (Ellefson and Carraway, 1976).

Livers were removed, weighed, and frozen in liquid nitrogen. Subsequently they were thawed, homogenized in ice-cold distilled water, and analyzed for total protein content and the activities of the enzymes aspartate aminotransferase (AST, E.C. 2.6.1.1), alanine aminotransferase (ALT, E.C. 2.6.1.2), lactate dehydrogenase (LDH, E.C. 1.1.1.27), and alkaline phosphatase (AP, E.C. 3.1.3.1) by methods previously described (Sitren and Stevenson, 1978).

Pancreata and small intestines were removed and processed for enzyme analysis. However, the results from this work will be reported in a separate paper.

The experiment was repeated once. Food consumption data from the two experiments showed that the intake of rats fed diets containing the raw meals was markedly lower than that of the other groups. Thus another experiment was carried out which included a restricted-fed (RF) casein group that was pair-fed to the group ingesting the raw peanut diet.

The protein efficiency ratio (PER) for each group was calcualted from the four week feeding period. The results from similarly treated groups of rats in all 3 experiments were not different and therefore the data have been pooled. Analysis of variance was performed and the means were compared at the 5% level of probability by the least significance difference test (Steel and Torrie, 1960).

RESULTS

In vitro studies

Table 2 lists the TI activity and lectin content of the flours. Raw peanut was found to contain the highest level of TI activity among all treatments. The application of dry heat led to a modest reduction while moist heat decreased it by approximately 80%. A similar response to heat was found with the soy flour. In all comparable treatments, the TI activity remained higher in peanut as compared with soy. The magnitude and response pattern of lectins in the flours paralleled that observed for TI activity.

The method of partially defatting the peanuts prior to further processing was low temperature pressing. This method did not generate sufficient heat to alter TI and lectin content, since samples of the raw, unpressed seeds of the same batch were analyzed and found to contain TI and lectin concentrations which were not higher than that found in the pressed product. Another variety of peanut, Virginia, was also analyzed and found to contain similar levels of TI and lectin.

In vivo studies

PER's are presented in Fig. 1. As expected, rats fed the raw or dry-heated soy diets showed markedly lower PER's than all other groups. The PER of rats fed the wet-heated soy approached 90% of the *ad libitum* (AL) casein control value. The PER's of the three groups ingesting the peanut diets were intermediate between the control and the raw soy fed groups. Apparently, wet heat processing of peanut did not improve its growth promoting ability. It should be noted that differences in PER values were not due to decreased food intake (Table 3) since the RF casein fed group had a PER similar to that of the AL casein control group although the latter group consumed one-third more food.

Table 1—Composition of diets

Ingredient	%
Protein (casein, peanut flour or soy flour)[a]	10
Sucrose	25
Total lipid[b] (corn oil + oil in flour)	8
Fiber	1
Vitamin mix AIN[c]	1
Mineral mix AIN[c]	3.5
Cholin bitartrate	0.2
Corn starch	to 100

[a] Casein was 89.6% protein, peanut 36.9% protein, and soy 35.3% protein by analysis; peanut and soy flours were added as raw, dry-heated (177°C for 20 min) or moist-heated (121°C for 20 min); the protein concentration of the completed diets differed slightly from one another but were isonitrogenous as verified by Kjeldahl analysis.
[b] Peanut flour was partially defatted; soy flour was full-fat; the lipid content of the flours was measured and taken into account in formulating the diets.
[c] American Institute of Nutrition (1977).

Hypotrophy of the liver occurred in all 3 peanut fed groups in comparison with the AL casein group (Fig. 2). In contrast, livers hypertrophied in the raw and dry heat soy groups but not in the wet heat soy group.

No significant differences in liver protein content were found among the groups (Fig. 2). However, several major differences were observed in hepatic enzyme activities (Fig. 3). ALT was significantly greater in all 3 peanut fed groups as compared with the AL control whereas no increase was noted in any of the soy fed groups. Significant changes in LDH were not found among or between the peanut and soy fed groups but all mean values were significantly less than the AL and RF casein groups. AP enzyme activities were significantly elevated in all soy and peanut fed rats, except for the raw peanut fed group, as compared with the AL casein control. However, the raw peanut fed group showed a significant increase when compared with the RF casein fed rats.

Serum chemistries are presented in Fig. 4. Total protein and albumin were significantly higher in both raw and dry-heated, but not wet-heated, peanut fed groups in comparison with both casein control groups. Conversely, a decrease was noted in the groups fed the raw and dry-heated, but not the wet-heated, soy diets. Serum urea N levels were elevated in all peanut and soy fed groups, however the increase was greatest in the groups consuming the raw and dry-heated legume diets. Rats eating the wet-heat peanut or
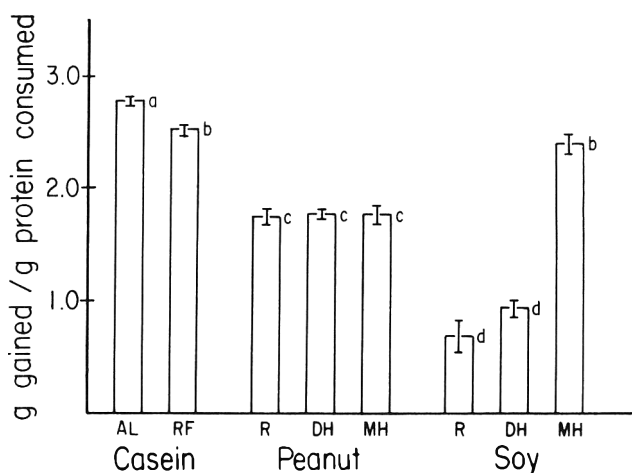

Fig. 1—Protein efficiency ratio (PER) of rats fed 10% dietary protein from raw or heat processed peanut or soy or from casein for 4 wk. AL = ad libitum fed, RF = food restricted to amount consumed by group R peanut, R = raw flour, DH = dry heated flour for 20 min at 177°C, MH = moist heated flour for 20 min at 121°C. Bars represent means ± SEM. Means with unlike superscripts are significantly different at P < 0.05.

Table 2—Trypsin inhibitor (TI) activity and lectin concentration of raw and processed peanut and soybean flours

Flour	Treatment	TI (% inhibition of enzyme)	Lectin (μg/g flour)
Peanut[a]	Raw	82	144.7
	Dry Heated[b]	76	117.7
	Moist Heated[c]	17	0.9
Soybean	Raw	76	112.1
	Dry Heated[b]	61	94.7
	Moist Heated[c]	11	0.0

[a] Partially defatted by cold pressing; lipid content of peanut and soy flours was similar.
[b] 177°C for 20 min.
[c] 121°C and 15 p.s.i. for 20 min.

soy diets had serum triglyceride levels which were not different from either of the control groups. Rats which ingested the raw or dry-heated peanut diets had increased triglycerides whereas animals which ate the dry heated soy showed a decrease (triglyceride levels were not determined in the raw soy diet group due to insufficient amounts of serum). The only significant difference observed in serum cholesterol was a decrease in rats fed the dry-heated soy diet.

DISCUSSION

THESE RESULTS confirm some of the reported affects of feeding peanut and soy (Liener, 1981; Borchers and Ackerson, 1950; Turner and Liener, 1975; Kakade et al., 1972) while extending our knowledge in other areas. Raw soy is known to contain biologically active antinutritional factors. However, it was an unexpected finding that peanut contained a higher TI and lectin content than soy when measured by identical extraction and chemical assay techniques. It is not possible to compare directly our figures for TI activities and lectin concentrations with those reported by other investigators. This is due to differences in extraction methods and assay techniques. We have defined lectin content in relation to purified lectin standards and TI activity in relation to a standard curve that was derived from the assay of purified soybean TI. Other workers have reported TI activity and lectin content in more arbitrary units (Borchers and Ackerson, 1950; Turner and Liener, 1975; Kakade et al., 1972). Comparisons can be made on the basis of the changes which occurred in TI and lectin content consequent to heat processing. For example, the application of moist heat for 20 min to soy was shown to lower both TI activity and lectin concentration by 90% or more (Turner and Liener, 1975; Liener, 1979). The figures obtained here for soy are an 86% decrease in TI and a 100% decrease in lectin concentration. Peanut showed a decrease of 79% in TI activity and of 99% in lectin content.

Although raw peanut and raw soy contain abundant TI and lectins which are mostly eliminated by moist heating, the biological response to feeding the raw and processed flours was not uniform either among or between the groups of rats. The differences in PER's among rats fed the three soy diets are in agreement with the findings of other investigators (Kakade et al., 1972; Liener, 1981; Rackis, 1974). However, there is still disagreement on whether or not the nutritive value of peanut is improved by heat processing. Cama and Morton (1950) and Balasundaron et al. (1958) claimed that moist heat-treated peanut improved PER, whereas Borchers and Acherson (1950) and Ananthara-man and Carpenter (1964) reported no improvement. Our results are in agreement with those of the latter two groups. This discrepancy may be explained by differences between laboratories in processing techniques as well as in the protein content of the diets and duration of feeding studies for the PER determinations. Thus, although raw peanut contains high levels of trypsin inhibitors and lectins as measured by in vitro assay (Table 2), they apparently do not exert a negative effect on growth, unlike that of soy, at a dietary protein concentration of 10%.

While most reports concerned with antinutritional factors of legumes have focused on their impact on the pancreas (Kwaan et al., 1968; Krogdahl and Holm, 1979; Liener, 1979), we thought it important to also examine their effects on other metabolic processes. As shown in Fig. 2 and 3, the liver was influenced greatly by feeding these legumes. However, the changes seen were not similar among groups fed the flours which were processed by identical methods. Feeding raw peanut decreased liver weight whereas raw soy increased it. The same trend was encountered with the dry heated products. However, feeding moist heated soy resulted in normal size livers while there was no change in the response to feeding moist heated peanut. Struthers et al. (1983) found no difference in liver weights between rats fed raw or moist heat-treated soy. However, they used rats which were much older than ours, and thus it is possible that the difference in maturity accounts for this discrepancy.
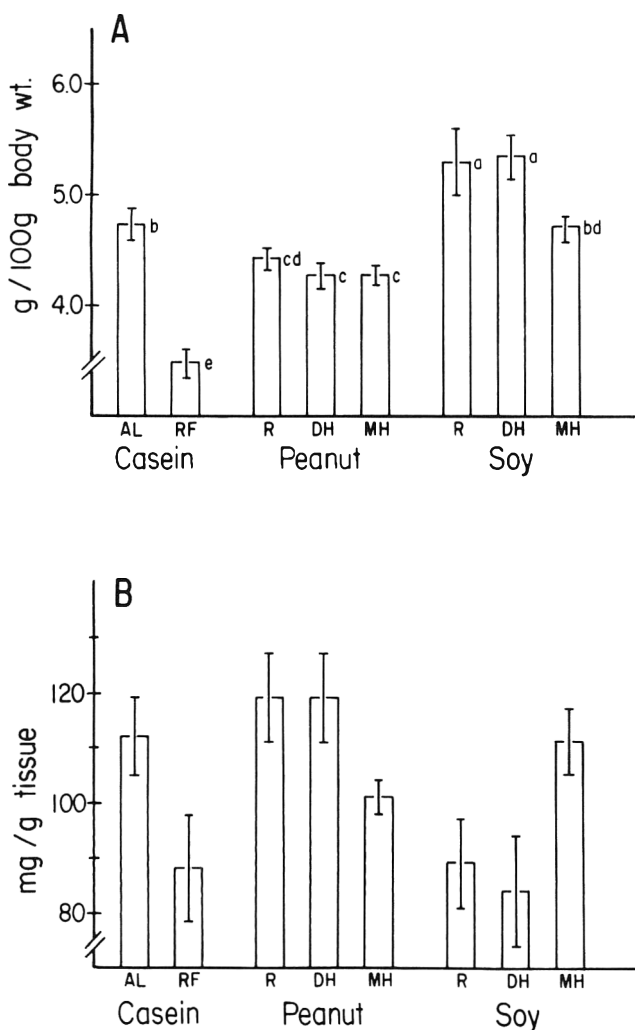

Fig. 2—Liver weight (A) and liver protein concentration (B) of rats fed 10% dietary protein from raw or heat processed peanut or soy or from casein for 4 wk. AL = ad libitum fed, RF = food restricted to amount consumed by group R peanut, R = raw flour, DH = dry heated flour for 20 min at 177°C, MH = moist heated flour for 20 min at 121°C. Bars represent means ± SEM. No significant differences were found among any of the groups.

Table 3—Average weekly food intake of rats

Dietary group	Week 1	2	3	4	Total
Casein ad libitum	64	75	83	93	315
Casein restricted fed[a]	48	61	59	67	236
Peanut					
Raw	48	61	59	67	236
Dry heated	47	67	60	62	236
Moist heated	55	61	69	66	251
Soy					
Raw	34	41	46	45	166
Dry heated	40	45	54	48	187
Moist heated	57	72	78	82	289

[a] Food intake in this group was restricted to the amount consumed by the group fed raw peanut.

The alterations seen in liver enzyme activities (Fig. 3) were generally consistent within, but not between, the two sets of experimental groups. Thus it is unlikely that these biochemical differences in liver function are related to antinutritional factor content since heat treatment reduced the levels of TI and lectin but had little effect on the liver tests. Furthermore these results provide no evidence of a correlation with PER values since, for example, no changes occurred among the 3 soy groups with respect to liver protein and enzyme activities even though PER's improved with dry and moist heating.

The observed differences in several of the serum chemistries (Fig. 4) did appear to be dependent upon the processing method. Rats fed the moist heated peanut and soy proteins, as opposed to the raw and dry heated products, showed serum levels which were nearer to those of the AL control (Fig. 4). Unexpectedly, the groups fed the raw and dry heated peanut had significantly higher total protein, albumin, and triglyceride concentrations than the groups ingesting the raw and dry heated soy.

This work does not necessarily demonstrate that the presence of TI and/or lectin was the agent(s) responsible for the observed findings. Other factors such as the effect of processing and digestibility of the proteins may be involved. For example, Liener (1979) showed that the removal of TI and lectin from raw soybean by affinity chromatography only partially overcame the growth depressing effect of intact raw soy. This suggests that the undenatured soy protein is in itself refractory to the digestive process unless first denatured by heat. This condition may not

apply to peanut protein since no improvement in growth efficiency occurred with heat processing. It is acknowledged that additional studies should be carried out in which TI and lectins are isolated and studied independent of the raw flour to determine their precise biological impact. Some of the results presented here do suggest that the physical and biochemical properties of antinutritional factors in peanut and soy differ considerably. Although raw peanut contains high TI activity as measured by its inhibitory action against bovine trypsin, it is conceivable that the chemical properties of peanut TI are such that it is suceptible to destruction by gastric digestion or unable to complex with, and inhibit, the trypsin secreted by rats. In any event, it is apparent that the nutritional properties of legumes cannot be classified according to the TI and lectin concentrations since peanut and soy have comparable levels yet rats tolerate raw peanut much better than raw soy.

It is possible that heat processing altered digestibility and/or amino acid availability which may have accounted for some of the results reported here. In general, improvements in digestibility and amino acid availability are associated with an improvement in the efficiency of utilization which in turn may be reflected by an increase in PER. However, as demonstrated in this study, PER's were not improved in rats fed processed peanut.

It is important to address the marked metabolic changes which occurred in the RF casein fed group. The most likely explanation is that they were consequent to an altered pattern of food intake by the rats rather than the lower amount of food eaten. Restricting a rat's intake of food alters its
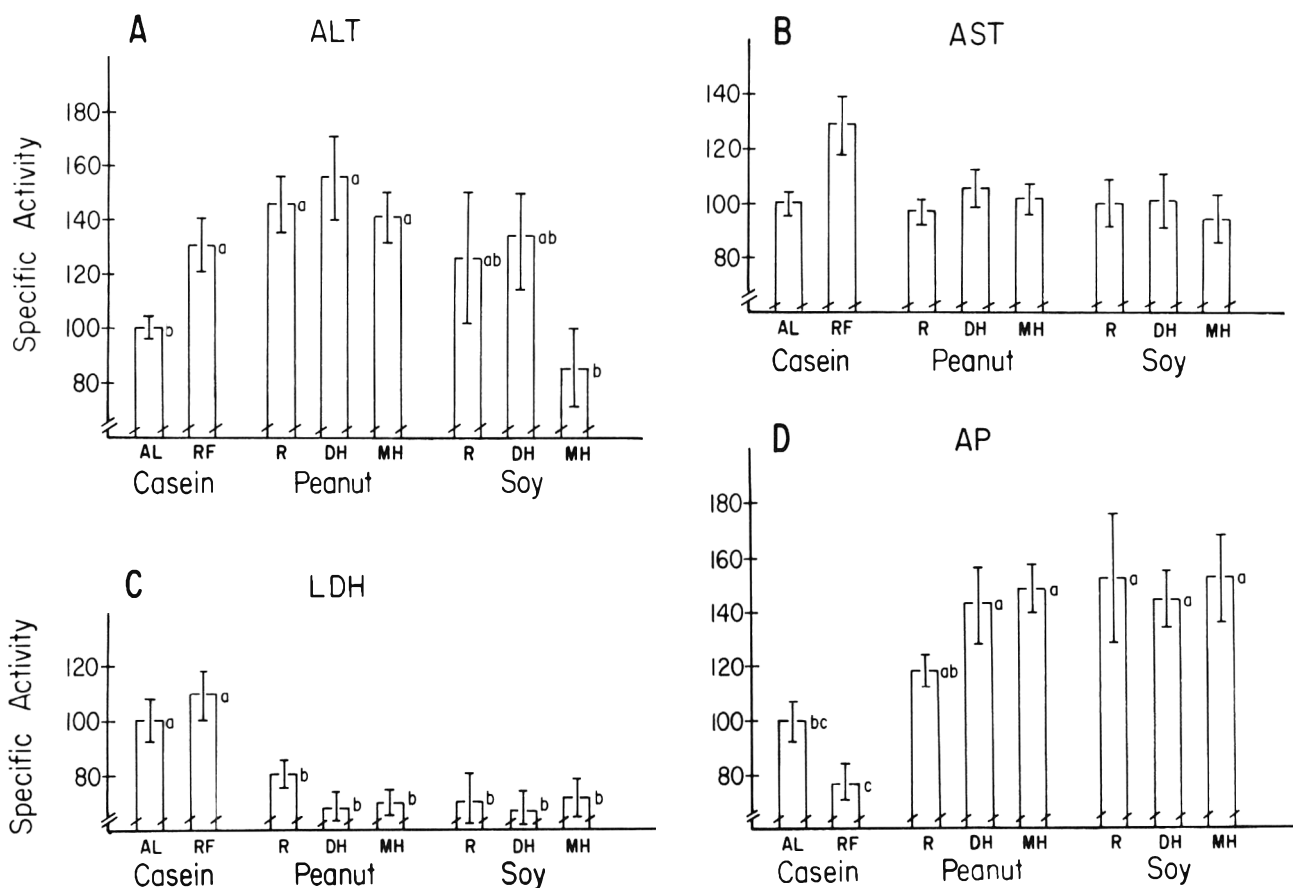
Fig. 3—Hepatocellular enzyme activities: A = alanine aminotransferase (ALT), B = aspartate aminotransferase (AST), C = lactate dehydrogenase (LDH) and D = alkaline phosphatase (AP), of rats fed 10% dietary protein from raw or heat processed peanut or soy or from casein for 4 wk. AL = ad libitum fed, RF = food restricted to amount consumed by group R peanut, R = raw flour, DH = dry heated flour for 20 min at 177°C, MH = moist heated flour for 20 min at 121°C. Results are expressed as a % of the AL casein group. Bars represent means ± SEM. Means with unlike superscripts are significantly different at P < 0.05.

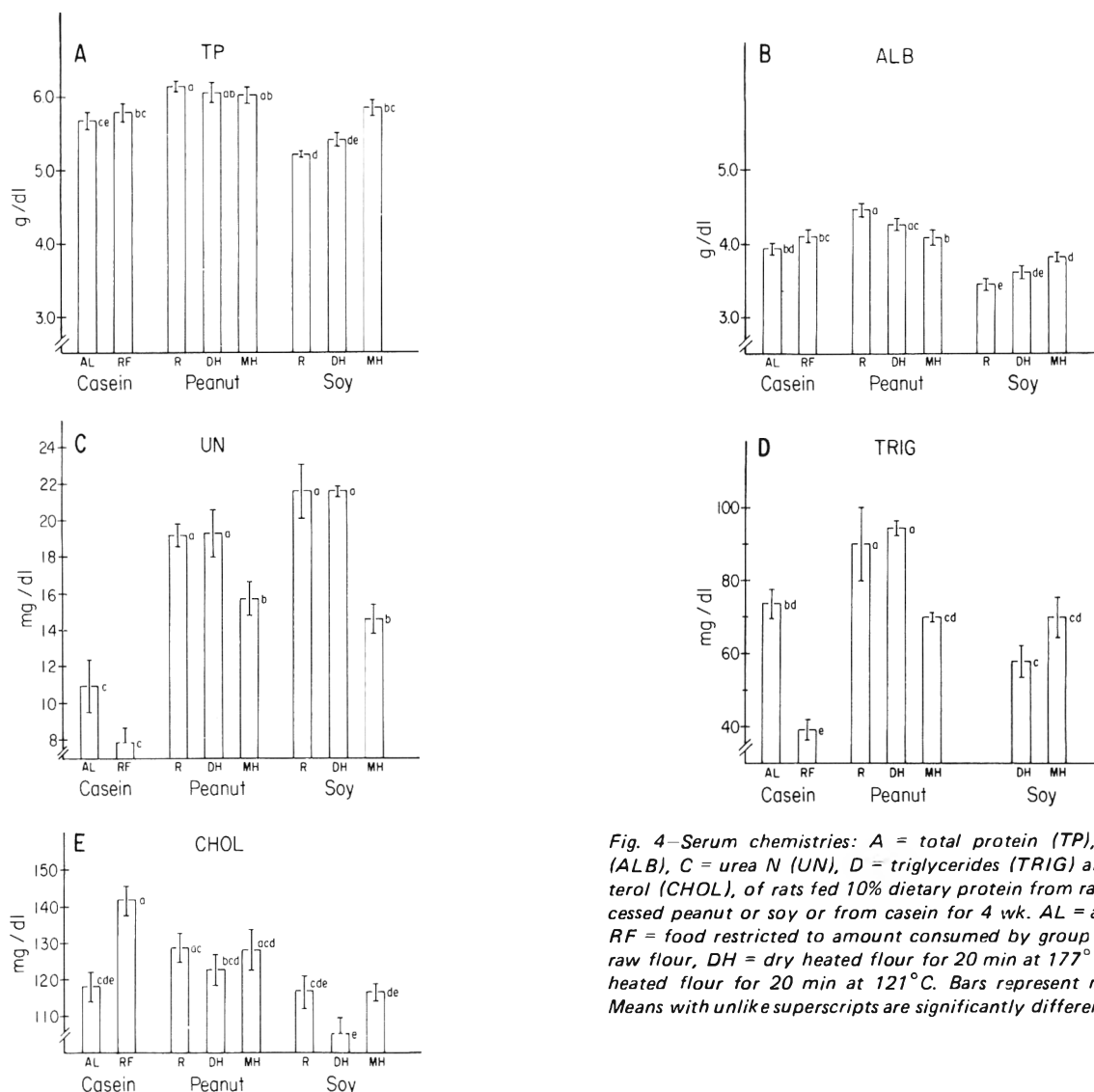*Fig. 4—Serum chemistries: A = total protein (TP), B = albumin (ALB), C = urea N (UN), D = triglycerides (TRIG) and E = cholesterol (CHOL), of rats fed 10% dietary protein from raw or heat processed peanut or soy or from casein for 4 wk. AL = ad libitum fed, RF = food restricted to amount consumed by group R peanut, R = raw flour, DH = dry heated flour for 20 min at 177°C, MH = moist heated flour for 20 min at 121°C. Bars represent means ± SEM. Means with unlike superscripts are significantly different at P < 0.05.*

feeding behavior from the normal *ad libitum* eating pattern. A rat which is provided with a surplus of food will eat small portions throughout the 24 hr day (LeMagnon and Talon, 1966). However, if the amount of food provided is restricted, the rat will adapt by eating virtually all the food in a shortened time period, thereby becoming a meal-eater (Cohn and Joseph, 1967). This adjustment occurs by as little as a 20% reduction in the quantity of food normally consumed (Cohn and Joseph, 1967). In comparison with *ad libitum* feeding, meal eating has been shown to cause marked deviations in body composition and in numerous metabolic functions (Furuya et al., 1979; Sitren and Stevenson, 1978). The RF casein fed rats in our study were observed to ingest their entire daily allowance of food within a few hours of presentation, and therefore they were without food for most of the day. In effect, they were, in all likelihood, fasting on the morning they were taken for analysis. These conditions would explain why this group showed substantial analytical differences from the AL casein controls.

REFERENCES

Ahmed, E.M., Sitren, H.S., and Schmidt, R.H. 1983. Determination of antinutrients in peanut and soybean seeds. Proc. South. Assoc. Agr. Sci. 20: 18.

American Institute of Nutrition. 1977. Report of the American Institute of Nutrition Ad Hoc Committee on standards for nutritional studies. J. Nutr. 107: 1340.

Anantharaman, K. and Carpenter, K.J. 1969. Effects of heat processing on the nutritional value of ground nut products. 1. Protein quality of groundnut cotyledons for rats. J. Sci. Food Agric. 20: 703.

Balasundaron, S., Cama, H.R., Makik, D.A., and Venkateshan, C. 1958. Nutritive value of differently processed ground nut meals and the effect of supplementation of the meals with amino acids, antibiotics and vitamin B_{12}. J. Nutr. 66: 75.

Borchers, R. and Ackerson, C.W. 1950. The nutritive value of legume seeds. 10. Effect of autoclaving and the trypsin inhibitor test for 17 species. J. Nutr. 41: 339.

Cama, H.R. and Morton, R.A. 1950. Changes occurring in the proteins as a result of processing groundnuts under selected industrial conditions 2. Nutritional changes. Brit. J. Nutr. 4: 297.

Cohn, C. and Joseph, D. 1967. Feeding frequency and lipogenesis in undernutrition. Can. J. Physiol. Pharmacol. 45: 609.

Doumas, B.T., Ard Watson, W., and Briggs, H.G. 1971. Albumin standards and the measurement of albumin with bromcresol green. Clin. Chim. Acta 31: 87.

Ellefson, R.D. and Caraway, W.T. 1976. Lipids and lipoproteins. Ch. 10. In "Fundamentals in Clinical Chemistry," (Ed.) N. Tietz. W.B. Saunders, Philadelphia, PA.

Furuya, S., Sitren, H.S., Ziegen, S., Offord, C.E., and Stevenson, N.R. 1979. Alterations in the circadian rhythmicity of rat small intestinal functions. J. Nutr. 109: 1962.

Herholzer, J.C. and Suggs, M.T. 1969. Standardized viral hemagglutination and hemagglutination-inhibition tests. Applied Micro. 18: 816.

Kakade, M.L., Simons, N.R., Liener, I.E., and Lambert J.W. 1972. Biochemical and nutritional assessment of different varieties of soybeans. J. Agr. Food Chem. 20: 87.

Kakade, L.M., Rackis, J.J., McGhee, J.E., and Puski, G. 1974. Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. Cereal Chem. 51: 376.

Krogdahl, A. and Holm, H. 1979. Inhibition of human and rat pancreatic proteinases by acid and purified soybean proteinase inhibitors. J. Nutr. 109: 551.

Kwaan, H.C., Kok, P., and Astrup, T. 1968. Impairment of growth and pancretic hypertrophy in rats fed trypsin inhibitor from raw peanuts. Experientia 24: 1125.

Le Magnon, J. and Talon, S. 1966. The spontaneous periodicity of ad libitum food intake in rats. J. Physiol. (Paris) 58: 323.

Liener, I. 1955. The photometric determination of the hemagglutinating activity of soyin and crude soybean extracts. Arch. Biochem. Biophys. 54: 223.

Liener, I. 1979. Significance for humans of biologically active factors in soybeans and other food legumes. J. Am. Oil Chem. Soc. 56: 121.

Liener, I.E. 1981. The nutritional significance of the plant lectins. In "Antinutrients and Natural Toxicants in Foods," (Ed.) R.L. Ory. Food and Nutrition Press, Wesport, CT.

Loton, R.E., Skutelsky, D., Danon D., and Sharon, N. 1975. The purification, composition and specificity of the anti-T lectin from peanut (Arachis hypogeae). J. Biol. Chem. 250: 8518.

Marsh, W.H., Fingerhut, B., and Miller, H. 1965. Automated and manual direct method for the determination of blood urea. Clin. Chem. 11: 624.

Rackis, J.J. 1974. Biolocial and physiological factors in soybean. J. Am. Oil Chem. Soc. 51: 161A.

Sitren, H.S. and Stevenson, N.R. 1978. The effects of mealfeeding at different times of the day on daily changes in serum insulin, gastrin, and liver enzymes in the rat. J. Nutr. 168: 1393.

Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics," Ch. 5. McGraw Hill, New York.

Struthers, B.J., MacDonald, J.R., Dahlgren, R.R., and Hopkins, D.t. 1983. Effects on the monkey, pig and rat of soy products with varying level of trypsin inhibitor and comparison with the administration of cholecystokinin. J. Nutr. 113: 86.

Turner, R.H. and Liener, I.E. 1975. The effect of selective removal of hemagglutinins on the nutritive value of soybeans. J. Agric. Food Chem. 23: 484.

Yatzidis, H. 1977. An improved biuret reagent. Clin. Chem. 23: 908.

Zlatkis, A., Zak, B., and Boyle, A.J. 1953. A new method for the direct determination of serum cholesterol. J. Lab. Clin. Med. 41: 486.

Ms received 7/7/84; revised 10/15/84; accepted 11/3/84.

James, W.P.T. and Theander, O. 1981. "The Analysis of Dietary Fiber in Food." Marcel Dekker, Inc., New York, NY.

Johansson, C., Aman, P., Asp, N., and Theander, O. 1982. Enzymatic and neutral detergent fibre methods for dietary fibre analysis. Swed. J. Agric. Res. 12: 157.

Marlett, J.A. and Lee, S.C. 1980. Dietary fiber, lignocellulose and hemicellulose contents of selected foods determined by modified and unmodified Van Soest procedures. J. Food Sci. 45: 1688.

Neilson, M.J. and Marlett, J.A. 1983. A comparison between detergent and nondetergent analyses of dietary fiber in human foodstuffs, using high-performance liquid chromatography to measure neutral sugar composition. J. Agric. Food Chem. 31: 1342.

Paul, A.A. and Southgate, D.A.T. 1978. "McCance and Widdowson's The Composition of Foods," 4th ed., p. 9. Elsevier/North Holland Biomedical Press, Amsterdam.

Rasper, V.F. 1981. Chemical and physical properties of dietary cereal fiber. Cereal Foods World 26: 228.

Robertson, J.B. and Van Soest, P.J. 1981. The detergent system of analysis and its application to human foods. Ch. 8. In "The Analysis of Dietary Fiber in Food," (Ed.) W.P.T. James and O. Theander, p. 123. Marcel Dekker, Inc., New York, NY.

Saeman, J.F., Moore, W.E., Mitchell, R.L., and Millett, M.A. 1954. Techniques for the determination of pulp constituents by quantitative paper chromatography. Tappi 37: 336.

Schaller, D.R. 1976. Analysis of cereal products and ingredients. Symposium on Food Fiber, AACC Annual Meeting, New Orleans.

Schweizer, T.F. and Wursch, P. 1981. Analysis of dietary fiber. Ch. 13. In "The Analysis of Dietary Fiber in Food," (Ed.) W.P.T. James and O. Theander, p. 203. Marcel Dekker, Inc., New York, NY.

Selvendran, R.R., Ring, S.G., and duPont, S.M. 1981. Determination of the dietary fiber content of the EEC samples and a discussion of the various methods of analysis. Ch. 7. In "The Analysis of Dietary Fiber in Food," (Ed.) W.P.T. James and O. Theander, p. 95. Marcel Dekkeer, Inc., New York, NY.

Slavin, J.L. and Marlett, J.A. 1983. Evaluation of high-performance liquid chromatography for measurement of the neutral saccharides in neutral detergent fiber. J. Agric. Food Chem. 31: 467.

Southgate, D.A.T. 1969. Determination of carbohydrates in foods. II. Unavailable carbohydrates. J. Sci. Food Agric. 20: 331.

Southgate, D.A'T. 1976. "Determination of Food Carbohydrates," p. 137. Applied Science Publishers, Ltd., London.

Southgate, D.A.T. 1981. Use of the Southgate method for unavailable carbohydrates in the measurement of dietary fiber. Ch. 1. In "The Analysis of Dietary Fiber in Food," (Ed.) W.P.T. James and O. Theander, p. 1. Marcel Dekker, inc., New York, NY.

Theander, O. and Aman, P. 1979a. The chemistry, morphology and analysis of dietary fiber. In "Dietary Fibers: Chemistry and Nutrition," (Eds.) G.E. Inglett and S.I. Falkehag, p. 215. Academic Press, New York, NY.

Theander, O. and Aman, P. 1979b. Studies on dietary fibres. Swedish J. Agric. Res. 9: 97.

Theander, O. and Aman, P. 1981. Analysis of dietary fibers and their main constituents. Ch. 5. In "The Analysis of Dietary Fiber in Food," (Eds.) W.P.T. James and O. Theander, p. 51. Marcel Dekker, Inc., New York, NY.

Weatherburn, M.W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Anal. Chem. 39: 971.

Ms received 8/13/84; revised 10/17/84; accepted 10/25/84.

Hafez, Y.S., Singh, G., McLellan, M.E., and Monroe-Lord, L. 1983. Effects of microwave heating soybean on nutritional quality of soybeans. Nutr. Rept. Intern. 28(2): 413.

Haines, P.C. and Lyman, R.C. 1961. Relationship of pancreatic enzyme secretion to growth inhibition in rats fed soybean trypsin inhibitor. J. Nutr. 74: 445.

Hill, P.R. and Harshburger, K.E. 1979. Effect of moisture content and amino acid supplementation of protein nutritive value of microwaved processed whole soybeans. Fed. Proc. 38: 238.

Hsu, H.W., Vavak, D.L., Satterlee, L.D., and Miller, G.A.C. 1977. A multienzyme technique for estimating protein digestibility. J. Food Sci. 42: 1269.

Kakade, M.C., Simons, M., and Linear, I.E. 1969. An evaluation of natural vs. synthetic substrates for measuring the antitryptic activity of soybean sample. Cereal Chem. 46: 518.

Kayamasbashi, H. and Lymar, R.L. 1966. Growth depression and pancreatic and intestinal changes in rats force-fed amino acid diets containing soybean trypsin inhibitor. J. Nutr. 89: 455.

Mai, J., Tasi, C.H., Arbruster, G., Chu, P., and Kinella, J. 1980. Effects of microwave cocking on food fatty acids, no evidence of chemical alteration or isomerization. J. Food Sci. 45: 1753.

McNaughton, J.C. and Reece, F.W. 1980. Effect of moisture content and cooking time on soybean meal urease index, trypsin inhibitor content and broiler growth. Poultry Sci. 29: 2300.

Mori, T., Nakamura, T., and Utsumi, S. 1981. Gelation mechanism of soybean 11S globulin: formation of soluble aggregates as transient intermediates. J. Food Sci. 47: 26.

Nitsan, Z. and Alumot, E. 1965. Overcoming the inhibition of intestinal proteolytic activity caused by raw soybean in chicks of different ages. J. Nutr. 84: 1979.

Nitsan, Z. and Bondi, A. 1965. Comparison of nutritional effects induced in chicks, rats and mice by soybean meal. Brit. J. Nutr. 19: 177.

Osman, S.A., Aboul Enein, A.M., Farage, R.S., and Mohamed, A.I. 1978. The change of fatty acid composition of blood and tissues of rats as influenced by induced hypercholesterolemia. The Arab J. Lab. Med. 2(3): 21.

Pour-El, A., Nelson, S.O., Peck, E., Tjhio, B., and Stetson, L.E. 1981. Biological properties of VHF and microwave-heated soybeans. J. Food Sci. 46: 880.

Pour-El, A. and Peck, E.E. 1973. Functionality of soybean protein as affected by heat processing presented at the symposium on quality improvement of the plant protein. 165th National Meeting of the American Chemical Society, Dallas, tx, April 9.

Rhee, K. and Rhee, K.C. 1981. Nutritional evaluation of the protein in oil seed products heated with sugar. J. Food Sci., 46: 164.

Schinogoethe, D.J. and Ahrar, M. 1979. Protein solubility, amino acid composition and biological value of regular and heated soybean and sunflower meal. J. Dairy Sci. 62: 925.

White, C.L., Green, D.E., Waldroup, P.W., and Stephenson, E.L. 1967. The use of untreated soybeans for chicks. Poultry Sci. 46: 1180.

Yamagishi, T., Tamarchi, F., and Shibasaki, K. 1980. Ioslation and partial characterization of heat-denatured products of soybean 11S globulin and their analysis by electrophoresis. Agric. Biol. Chem. 44: 1575.

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Iron Binding by Fiber is Influenced by Competing Minerals

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──────────── ABSTRACT ────────────

Iron was bound by neutral (NDF) and acid (ADF) detergent fibers extracted from cooked pinto beans (*Phaseolus vulgaris*). Soluble iron (total iron minus bound iron) in the presence of NDF was increased from 13.11 ± 5.08 to $35.58 \pm 9.20\%$ and from 22.22 ± 164 to $29.98 \pm 0.96\%$ when 1.17 and 1.38 ppm (18.4 and 21.4 μM) of copper and zinc were added, respectively. In contrast, 0.93 ppm (14.6 μM) of copper decreased the soluble iron from 61.02 ± 5.77 to $17.88 \pm 4.5\%$ in the presence of ADF. Neither magnesium or cobalt altered the amount of soluble iron in the presence of fiber. The change in soluble iron was directly proportional to the concentration of copper and zinc. The presence of two types of binding sites is proposed.

INTRODUCTION

IRON BIOAVAILABILITY varies within foods and depends on the concentration of enhancers and inhibitors of iron absorption. Generally high iron bioavailability is observed from foods such as meat while low bioavailability is observed from cereals and legumes (Layrisse et al., 1969). Poor iron bioavailability in cereals and legumes seems to be associated with the presence of compounds capable of forming insoluble complexes with iron, such as phytates and fiber (Reinhold et al., 1981; Oberleas et al., 1966). Reinhold et al. (1975) reported that fiber was responsible for the low iron bioavailability observed from cereals. However, different investigators have found conflicting results when studying the effect of fiber on mineral metabolism. Simpson et al. (1981) found that addition of wheat bran to high and low iron bioavailability diets decreased iron absorption, whereas Sandstead et al. (1978) found that addition of corn and wheat bran to the diet had no effect on iron balance of men. Some investigators have shown fiber inhibits iron absorption (Kelsay et al., 1978; Reinhold et al., 1975; Garcia and Wyatt, 1982; Fernandez and Phillips, 1982a) while others have shown no effect (Miller, 1979; Kelsay et al., 1979).

The effect of fiber on iron absorption may depend not only on the amount of fiber eaten but also on the source or type of fiber consumed. Often overlooked is the possible presence of inhibitors of iron binding by fiber, which in turn would increase the amount of available iron. This has gained recent interest as some researchers have reported that compounds such as phytate, citric acid, ascorbic acid, EDTA and others inhibit iron binding by fiber (Reinhold et al., 1981; Fernandez and Phillips, 1982b). However, few have studied the effect of competing minerals on the ability of fiber to bind iron.

The purpose of this investigation was to evaluate the effect of the competing minerals, copper, zinc, magnesium and cobalt on the iron binding capacity of fiber.

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MATERIALS & METHODS

Sample preparation

Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were extracted from cooked pinto beans (*Phaseolus vulgaris*) obtained locally. The beans were cleaned and washed with tap water and soaked in glass-distilled demineralized water for 24 hr at 24°C (3:1 water to bean ratio). After soaking, the water was discarded and the beans were boiled in demineralized water until soft (70 min). The beans were cooled and the supernatant discarded. Beans were blended to a paste (Waring Blendor), frozen and freeze-dried using a Virtis Model 10-100 Lyophilizer. The dried bean powder was reground (Salton Quick-Mill) to pass through a 60 mesh screen and stored at –40°C until used.

NDF was obtained by the method of Robertson and Van Soest (1977) as modified by Reinhold and Garcia-Lopez (1979). ADF was obtained using the method of Van Soest (1973).

Binding

The effect of copper, zinc, magnesium and cobalt on iron binding by fiber was determined using a method described by Reinhold et al. (1981). Fiber samples were ground using a mortar and pestle to pass a 60 mesh screen. Ten milligrams of either NDF or ADF were weighed into separate test tubes of 14 mL capacity and washed for 20 min with 10 mL glucose-saline solution (GSS). The GSS contained 128 mM NaCl, 4 mM KCl and 28 mM D-glucose. After washing the samples were centrifuged for 10 min at 705 x g and the supernatant discarded. Ten milliliters glucose-saline mineral solution (GSMS) containing 0.65 ppm (11.64 μM) iron and different amounts of a second mineral (Cu, Zn, Mg or Co) were added to the fiber residue. The GSMS contained 128 mM NaCl, 4 mM KCl, 28 mM D-glucose, 1 mM sodium acetate, 0.5 mM imidazole, 0.2 mM HCl, 0.028 mM ascorbate and the desired concentration of minerals. This level of ascorbate was shown to have no influence on iron binding (Reinhold et al., 1981) and was present to stabilize aqueous ferrous iron. The tubes were mixed at 21 oscillations/min using a tube rocker for 20 min and the pH of each solution was adjusted to 6.50 ± 0.05 with 0.02N HCl or 0.02N NaOH. In experiments with copper the pH was first adjusted using 0.1N then 0.02N NaHCO₃ to avoid precipitation. Mixing was continued for 40 min and the tubes were centrifuged again for 10 min at 705 x g. The supernatants were removed and the mineral concentration determined using a flame-type Perkin Elmer Model 2380 atomic absorption spectrophotometer.

For each solution treated with fiber, a control was prepared from which fiber was omitted. The difference in iron concentration between the control solution and that exposed to fiber measured the extent of iron bound by fiber. The ability of Cu, Zn, Mg, and Co to inhibit iron binding by fiber was measured by determining the percent of soluble iron as follows:

$$\% \text{ soluble iron} = \frac{\text{total iron} - \text{bound iron}}{\text{total iron}} \times 100$$

The source of minerals as well as the range of concentrations used are shown in Table 1. The data were analyzed by analysis of variance and Duncan's multiple range test using a MINITAB statistical software package (Ryan et al., 1982).

RESULTS & DISCUSSION

THE EFFECTS of copper, zinc, magnesium and cobalt on the iron bound by NDF are shown in Fig. 1. Copper and zinc significantly increased the percent soluble iron, this decreased the iron bound by NDF. Copper increased (p < 0.01) the soluble iron from 13.11 ± 5.08 with no copper

Table 1—Sources of minerals and range of concentrations tested

Mineral	Source	NDF[a] (ppm)	ADF[a] (ppm)
Fe	$FeNH_4(SO_4)_2 \cdot 12 H_2O$	0.65	0.65
Cu	$CuSO_4 \cdot 5H_2O$	0 – 1.17	0 – 0.93
Mg	$MgSO_4 \cdot 7H_2O$	0 – 1.00	0 – 0.47
Zn	$ZnSO_4 \cdot 7H_2O$	0 – 1.40	0 – 0.93
Co	$CoCl_2 \cdot 6H_2O$	0 – 1.00	–

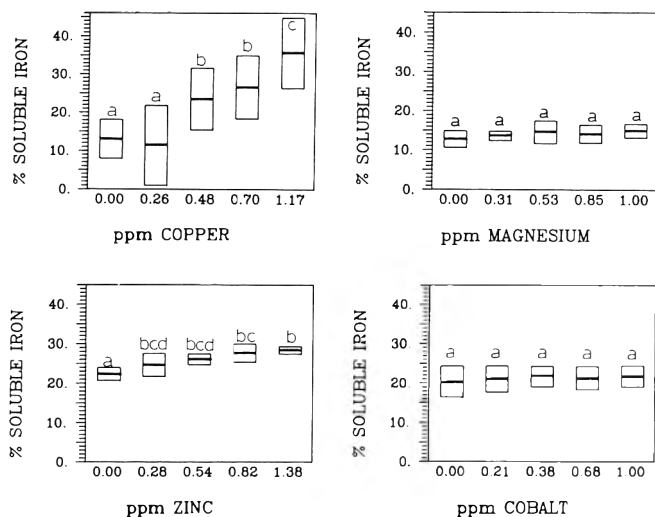[a] NDF = neutral detergent fiber; ADF = acid detergent fiber.

Fig. 1—Effect of copper, magnesium, zinc and cobalt on soluble iron in the presence of neutral detergent fiber (NDF). Bars with different letters along the same mineral are different at p < 0.01. Each bar represents the mean of two duplicates. The unshaded area is plus or minus one standard deviation.

Fig. 2—Effect of copper, magnesium and zinc on soluble iron in the presence of acid detergent fiber (ADF). Bars with different letters along the same mineral are different at p < 0.01. Each bar represents the mean of two duplicates. The unshaded area is plus or minus one standard deviation.

Fig. 3—Varying levels of copper or zinc bound by 10 mg NDF or 10 mg ADF in the presence of 0.65 ppm iron. Each point represents the mean ± 1 SD of two duplicates.

added to 35.58 ± 9.20% when 1.17 ppm (18.4 μM) of copper was present. Although zinc also increased (p < 0.01) the soluble iron, the increment was smaller than that obtained with copper (from 22.22 ± 1.64% with no zinc added to 28.88 ± 0.96% when 1.38 ppm (21.4 μM) of zinc were present). Neither magnesium nor cobalt increased or decreased the soluble iron when present up to concentrations of one ppm (41.1 and 17.0 μM, respectively). This lack of measurable effect with magnesium may be due to the high concentration of magnesium already present in the NDF fraction (18.33 ± 8.25 ppm). Although no other calcium data are available for comparison, Berner and Hood (1983) reported a decrease in the amount of iron bound by sodium alginate when calcium was added. Our observations are consistent with those of Thompson and Weber (1982), who found a decrease in the amount of copper and zinc bound by a different source of NDF when added together.

The effect of copper, zinc and magnesium on soluble iron in the presence of ADF is shown in Fig. 2. Opposite to what was observed with NDF, copper decreased the percent of soluble iron significantly. The percent of soluble iron was decreased (p < 0.01) from 61.02 ± 5.77% with no copper to 17.88 ± 4.50% when 0.93 ppm of (14.6 μM) copper was present. Neither magnesium nor zinc changed the percent of soluble iron.

The increase in iron binding by ADF as opposed to the decrease in iron bound by NDF in the presence of copper may be due in part to a concentration effect. The concentration of iron in the ADF fraction was lower than the NDF fraction (15.93 ± 12.00 vs. 5.31 ± 3.26 ppm of iron). However, such differences were not statistically significant due to wide variations in endogenous iron content. It is impor-

tant to note that in all the experiments the ADF fraction bound less iron than the NDF fraction.

The absence of hemicellulose in the ADF fraction as well as other unidentified compounds (possibly polyphenolic compounds) seems to uncover a binding site for copper which synergistically enhances iron binding. This binding site appears to be unaffected by zinc. This is corroborated by the fact that ADF bound more copper and zinc than NDF at all concentrations tested (Fig. 3).

It is also possible that lignin-type compounds formed during boiling may have generated additional binding sites in the ADF. These data suggest the presence of two types of chemically distinct binding sites: site type A which is specific for Cu and/or Zn and site type B that binds Cu, Zn and Fe. Site type B seems to be associated with the hemicellulose fraction or possibly with polyphenolic com-

Bioavailability of Iron in Green Peas, Spinach, Bran Cereal, and Cornmeal fed to Anemic Rats

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────────ABSTRACT────────

Bioavailability of iron from green peas, spinach, bran cereal and cornmeal was evaluated by hemoglobin regeneration efficiency in the anemic rat. By analysis these respective food items contained 85, 163, 55 and 35 mg of iron per kg of dry weight. Iron from ferrous sulfate, peas, spinach, bran cereal and cornmeal was converted to hemoglobin iron with respective efficiencies of 67, 64, 34, 53 and 39 percent. Based on these bioavailabilities, peas, spinach, bran cereal, and cornmeal provide 14, 19, 12, and 3 mg of available iron per 1000 kcal. Plant foods can make significant contributions of available iron in the diet.

INTRODUCTION

A MAJOR NUTRITION PROBLEM for several segments of the population is iron deficiency anemia (Ten-State Nutrition Survey, 1968-70). Although iron fortification has resulted in increased intake of food iron, some sources of iron used in fortification are of low bioavailability (Rios, 1975). Food iron occurs in two forms with respect to the mechanism of absorption: heme and nonheme iron (Hallberg and Bjorn-Rassmussen, 1972; Cook, 1983). Heme iron is readily absorbed and its absorption is little affected by the other ingredients in foods. Nonheme iron is absorbed less efficiently and its absorption is vulnerable to various factors in foods (Cook, 1983). The factors in food which enhance or inhibit nonheme iron absorption have recently been reviewed (Monson et al., 1978; Morris, 1983; Mahoney and Hendricks, 1984).

Plant foods are widely consumed and contribute a major percentage of the iron in human diet. Finding the plant foods which are rich in available iron is important for meeting the iron requirement of the population without iron fortification or supplementation. These studies were designed to examine the bioavailability of the iron in green peas, spinach, bran cereal and cornmeal. Hemoglobin regeneration by anemic rats was used to estimate iron bioavailability (Mahoney and Hendricks, 1982).

MATERIALS & METHODS

Foods

The foods used in these experiments were purchased from local supermarkets. Frozen green peas and spinach were freeze-dried and ground in a stainless steel blender. Bran cereal and enriched corn meal were commercial products and also finely ground in a stainless steel blender. The iron concentration in the foods was estimated by AOAC (1980) method 14,011.

Diets

The composition of the diets is shown in Table 1. The foods were added to a basal diet formulation to provide 20 mg iron/kg above the basal diet mix. The amounts of dietary protein, fat, fiber, phosphate, calcium, sodium and potassium were then formulated to be similar in all diets by adjusting the levels of casein, corn oil,

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cellulose, NaH_2PO_4, $CaCO_3$, NaCl or KCl respectively. Freshly purchased $FeSO_4 \cdot 7H_2O$ was used as a reference source of dietary iron. Diet ingredients were thoroughly mixed in a stainless steel mixer bowl by a mechanical mixer.

Animal experiment

Male, weanling, Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) were individually housed in stainless steel cages with wire-mesh bottoms and fronts. Housing was in a temperature controlled room with a 12-hr day and night lighting cycle. The rats were made anemic by feeding the basal diet without any added $FeSO_4 \cdot 7H_2O$ (9.76 ppm iron) for 7 days and taking approximately 1 mL of blood from the retro-ocular capillary bed on day 1 and again on day 4. The anemic rats were randomly assigned to groups of 9 or 10 animals each balancing across treatments for hemoglobin concentration and body weight. The animals reported in this study consuming peas as the primary iron source were fed in a separate trial in which initial hemoglobin levels were lower. All studies follow this laboratory follow a similar protocol and have $FeSO_4$ and basal groups to check relative iron bioavailability and to compare completeness of depletion. Thus between trial comparisons show low variability within a single food iron source.

During the 10-day repletion period, each rat received 9g daily of the respective diets. Any spilled diet was weighed and recorded to determine the net diet consumption, used for determining iron intake. Demineralized water was allowed ad libitum.

Analytical procedures

At the initiation and termination of the experiment, blood was obtained from the retro-ocular capillary bed with a heparinized glass capillary tube. Hemoglobin was quantitated from duplicate samples of blood by colorimetry (Crosby et al., 1954). Food, diet

Table 1—Diet formulations fed to rats (gm/kg)

Item	$FeSO_4$	Peas	Spinach	Bran cereal	Cornmeal
Peas		240			
Spinach			120		
Bran cereal				370	
Cornmeal					570
Casein	198	134	155.8	146.7	148.2
Corn oil	100	100	96.4	88.9	93.2
Cellulose	50	50	42.8	21.1	46.6
NaH_2PO_4	26.9	26.9	25.5	11.5	25.9
$CaCO_3$	17.0	17.0	14.8	10.6	17.1
Vitamin mixture[a]	20	20	20	20	20
Mineral mixture[b]	11.6	11.6	11.6	11.6	11.6
NaCl	2.5		1.0		3.0
KCl	10.8			3.2	9.5
$FeSO_4 \cdot 7H_2O$	0.1				
Dextrose	563.1	400.5	512.1	310.4	54.9
Fe content, mg/kg[c]	29.2	30.8	30.7	35.4	29.7

[a] The vitamin mixture contained (in g/kg): alpha-tocopherol (1000 IU/g), 5.0; L-ascorbic acid, 45.0; choline chloride, 75.0; D-calcium pantothenate, 3.0; inositol, 5.0; menadione, 2.25; niacin, 4.5; PABA, 5.0; pyrodoxine-HCl, 1.0; riboflavin, 1.0; thiamin-HCl, 1.0; vitamin A acetate, 900,000 units; calciferol (D_2), 100,000 units; biotin, 20 mg; folic acid, 90 mg; vitamin B_{12}, 1.35 mg; and dextrose added to make to 1 kg.
[b] The mineral mixture contained (in g/kg): KCl, 296.7; $MgCO_3$, 121; $MnSO_4$, 12.7; $CoCl_2 \cdot 6H_2O$, 0.7; $ZnSO_4 \cdot 7H_2O$, 38; $CuSO_4 \cdot 5H_2O$, 1.6; KI, 0.8; $NaMoO_4 \cdot 2H_2O$, 0.12; and glucose, 528.4.
[c] Iron content of the diets as determined by analysis.

Item	Basal[a]	FeSO$_4$	Peas	Spinach	Bran cereal	Cornmeal	F	LSD
Number of rats	10	10	9	10	10	10	—	
Initial BW, g	88	92	89	92	91	87	NS	—
BW gain, g	27	24	24	21	26	26	2.50	4.7
Initial hemoglobin, g/dL	7.31	7.30	6.0	7.22	7.28	7.29	NS	—
Hemoglobin gain, g/dL	0.39	5.28	5.72	2.40	4.73	2.33	51.93	0.63/.84
Iron intake, mg	0.86	2.61	2.79	2.76	3.18	2.66	NS	—
Efficiency, %	63	67	64	34	53	39	50.29	5.48/7.30
Relative efficiency	94	100	96	51	79	58	—	—

[a] The data from the rats fed the basal diet were not included in the statistical analysis.
[b] Mean differences must equal or exceed the Least Significant Difference values to be statistically significant at the 5 or 1 percent levels of probability.

and liver samples were wet-ashed in 800 mL Kjeldahl flasks using H_2SO_4, HNO_3, and H_2O_2 as oxidants. Samples were left in about 4 mL H_2SO_4 solution and diluted to 50 mL using demineralized water. Iron concentration of the ash solution was determined color-imetrically (AOAC, 1980). Hemoglobin regeneration efficiency was calculated for each animal as follows:

$$\text{Efficiency} = \frac{\text{mg HbFe(final)} - \text{mg HbFe(initial)}}{\text{mg iron consumed}} \times 100$$

Hemoglobin iron (mg) was calculated as follows:

$$\text{mg Hb iron} = \text{g body Wt} \times \frac{0.067 \text{ mL blood}}{\text{g body Wt}} \times \frac{\text{g hemoglobin}}{\text{mL blood}} \times \frac{3.35 \text{ mg Fe}}{\text{g Hb}}$$

Mg of iron consumed was calculated from (food given − food not eaten) × analyzed iron value for that diet.

The data were analyzed statistically by analysis of variance. When F was significant ($P < 0.05$), least significant difference values (LSD) were calculated (Steele and Torre, 1960; Carma and Swanson, 1973).

RESULTS & DISCUSSION

THE IRON CONTENT of the foods used were 85, 163, 55, and 35 mg/kg for peas, spinach, bran cereal and cornmeal, respectively, on a dry weight basis. On a fresh basis these values would be 1.6, 1.4, 5.3 and 3.1 mg Fe per 100g for the foods. These values are in agreement with published values (Watt and Merrill, 1967).

The hematinic responses of the rats are presented in Table 2. The efficiency of converting iron from the ferrous sulfate diet into hemoglobin by the anemic rats was 67%. This value is similar to what Park et al. (1983a, 1983b) reported for ferrous sulfate and to what we have observed in other experiments.

Uncooked peas were a good source of available iron with an efficiency 96% that of ferrous sulfate (Table 2). Smith and Otis (1937) reported that the iron in dried peas is also well utilized by iron deficient rats with bioavailabilities between 85 and 88% that of ferric chloride.

The iron uncooked spinach was utilized only 51% as efficiently as in ferrous sulfate (Table 2). Gordon and Chao (1984) using similar methodology obtained a relative bio-availability of 75% for spinach compared with ferrous sulfate using this procedure. A relative bioavailability of 67% for spinach compared with ferric chloride was calculated from the rat data of Pye and MacLeod (1946) in which the methodology was similar to that used in Table 2. Van Campen and Welch (1980) reported that iron deficient rats absorbed 69.7 or 69.8% of the Fe-59 dosed in single meals when given as intrinsically labelled uncooked lyophilized spinach or ferric chloride. Thus, iron bioavailability as

Table 3—Iron content and potential iron availability from green peas, spinach, bran cereal and cornmeal

	Green peas	Spinach	Bran cereal	Cornmeal
Fe Content, mg/100g fresh basis	1.6	1.4	5.3	3.1
Fe Content, mg/1000 kcal	22.0	57.0	17.0	17.0
Available Fe, mg/100g[a]	1.1	0.5	2.8	1.2
Available Fe, mg/1000 kcal	14.0	19.0	12.0	3.3
Available Fe, mg/serving[b]	0.9	0.5	0.8	0.2
Estimated calories/serving	55.0	24.0	71.0	73.0

[a] Available iron calculated by multiplying the iron content of the food as analyzed by the efficiency values obtained with the rat studies.
[b] Servings of green peas, spinach and cornmeal are based on 1/2 cup of the prepared food. Serving of the bran cereal was calculated as 28g.

determined in this study (Table 2) appears to be lower than previously reported by others.

The bioavailability of the iron in bran cereal relative to ferrous sulfate was 79% (Table 2). The bioavailability of iron in wheat bran relative to ferrous sulfate was reported to be 97% (Gordon and Chao, 1984) and 98% relative to ferric chloride calculated from the data of Pye and Mac-Leod (1946). Others have reported that the bioavailability of iron in wheat bran is similar to ferric chloride when fed to rats (Rose and and Vahlteich, 1932; Vahlteich et al., 1936; Free and Bing, 1940). This is because rats contain intestinal phytase (Pileggi, 1959) which is thought to free any phytate bound iron, thus making it more bioavailable (Morris and Ellis, 1976).

The iron in cornmeal was 58% as available as ferrous sulfate (Table 2). However, Smith and Otis (1937) reported that iron in ground whole corn was 92 and 96% as available to rats as ferric chloride. Ifon (1981) reported that in rats 37.5% of the iron ingested from maize was utilized for hemoglobin regeneration. This is similar to the efficiency found in the present study.

Iron deficiency anemia can be corrected by the ingestion of foods from which the iron is available for absorption and metabolism provided other hematinic factors such as copper, folic acid and cobalamin are also adequate in the diet. The foods used in this study contribute significant quantities of available iron to the human diet (Table 3). This contribution of available iron would be accompanied by a very low calorie intake.

It is concluded that plant foods can be excellent sources of dietary iron. Both the total amount of iron present in

the food and its bioavailability need to be considered when evaluating foods as sources of iron to be included into the diet. The bioavailability data reported here were obtained in individual food sources; but, foods are combined in many ways both qualitatively and quantitatively in different menus. The interactions of foods as they affect iron bioavailability need to be explored as is being done for meat enhancement of nonheme iron bioavailability (Monson et al., 1978; Shah et al., 1983; Ranger and Neale, 1984).

REFERENCES

AOAC. 1980. "Official Methods of Analysis", 13th ed., (Ed.) W. Horwitz. Association of Official Analytical Chemists, Washington, DC.

Carma, S.G. and Swanson, M.R. 1973. An evaluation of ten pairwise multiple comparison procedures by Monte Carlo methods. J. Am. Stat. Assoc. 68: 66.

Cook, J.D. 1983. Determinants of nonheme iron absorption in man. Food Technol. 37(10): 124.

Crosby, W.H., Munn, S.I., and Furth, F.W. 1954. Standardized method for clinical hemoglobinometry. U.S. Armed Forces Med. J. 5: 693.

Free, A.H. and Bing, F.C. 1940. Wheat as a dietary source of iron. J. Nutr. 19: 449.

Gordon, D.T. and Chao, L.S. 1984. Relationship of components in wheat bran and spinach to iron bioavailability in the anemic rat. J. Nutr. 114: 526.

Hallberg, L. and Bjorn-Rasmussen, E. 1972. Determination of iron absorption from whole diet. A new two-pool model using two radioiron isotopes given as haem and nonhaem iron. Scand. J. Haematol. 9: 193.

Ifon, E.T. 1981. Bioavailability to rats of the iron contents in selected cereals and pulses. Nutr. Rpts. Intl. 24: 25.

Mahoney, A.W. and Hendricks, D.G. 1982. Efficiency of hemoglobin regeneration as a method of assessing iron bioavailability in food products. In "Nutritional Bioavailability of Iron", p. 1, (Ed.) C. Kies. American Chemical Society, Washington, DC.

Mahoney, A.W. and Hendricks, D.G. 1984. Potential of the rat as a model for predicting iron bioavailability for humans. Nutrition Research (In press).

Monson, E.R., Hallberg, L., Layrisse, M., Hegsted, D.M., Cook, J.D., Mertz, W., and Finch, C. 1978. Estimation of available dietary iron. Am. J. Clin. Nutr. 31: 134.

Morris, E.R. and Ellis, R. 1976. Isolation of monoferric phytate from wheat bran and its biological value as an iron source to the rat. J. Nutr. 106: 753.

Morris, E.R. 1983. An overview of current information on bioavailability of dietary iron to humans. Federation proceedings 42: 1716.

Park, Y.W., Mahoney, A.W., Cornforth, D.C., Collinge, S.K., and Hendricks, D.G. 1983a. Bioavailability to anemic rats of iron from fresh, cooked or nitrosylated hemoglobin and myoglobin. J. Nutr. 113: 680.

Park, Y.W., Mahoney, A.W., and Hendricks, D.G. 1983b. Bioavailability of different sources of ferrous sulfate iron fed to anemic rats. J. Nutr. 113; 2223.

Pileggi, V.J. 1959. Distribution of phytase in the rat. Arch. Biochem. Biophys. 80: 1.

Pye, O.F. and MacLeod, G. 1946. The utilization of iron from different foods by normal young rats. J. Nutr. 32: 677.

Ranger, C.R. and Neale, R.J. 1984. Iron availability from soy, meat and soy/meat samples in anemic rats with and without prevention of coprophagy. J. Food Sci. 49: 585.

Rios, E. 1975. The absorption of iron as supplements in infant cereal and infant formulas. Pediatrics 56: 686.

Rose, M.S. and Vahlteich, E.M. 1932. Factors in food influencing hemoglobin regeneration. 1. Whole wheat flour, white flour, prepared bran, and oatmeal. J. Biol. Chem. 96: 593.

Shah, B.G., giroux, A., and Belonje, B. 1983. Bioavailability of iron in ground beef and plant protein concentrates. Nutr. Res. 3: 547.

Smith, M.C. and Otis, L. 1937. Hemoglobin regeneration in anemic rats in relation to iron intake. J. Nutr. 13: 573.

Steel, R.G.D. and Torrie, J.H. 1960. "Principles and Procedures of Statistics," p. 99. McGraw-Hill Book Co., Inc., New York.

Ten-State Nutrition Survey, 1968-69. 4. Biochemical. Department Health, Education and Welfare Publication No. 72-8132.

Vahlteich, E.M., Rose, M.S., and MacLeod, G. 1936. The effect of digestibility upon the availability of iron in whole wheat. J. Nutr. 11: 31.

Van Campen, D.R. and Welch, R.M. 1980. Availability to rats of iron from spinach: Effects of oxalic acid. J. Nutr. 110: 1618.

Watt, B.K. and Merrill, A.L. 1967. "Composition of Foods. Raw, Processed, Preserved." Agriculture Handbook No. 8. Agricultural Research Service, U.S. Dept. of Agriculture, Washington, DC.

pounds. NDF has been reported to contain such compounds (Garcia Lopez and Wyatt, 1981). Site type A seems to be associated with the cellulose-lignin fraction. Reinhold et al. (1981) reported the presence of one type of binding site in NDF obtained from corn and wheat. However, Fernandez and Phillips (1982b) reported the presence of two types of binding sites for iron in lignin.

This investigation has shown decreased iron binding by NDF in the presence of copper and zinc, whereas an increase in iron binding by ADF was shown in the presence of copper. This suggests multiple binding sites for these minerals. These results demonstrate the need to consider competitive binding by other minerals as well as the source of fiber when assessing the effect of fiber on iron metabolism.

REFERENCES

Berner, L.A. and Hood, L.F. 1983. Iron binding by sodium alginate. J. Food Sci. 46: 755.

Fernandez, R. and Phillips, S.F. 1982a. Components of fiber impair iron absorption in the dog. Am. J. Clin. Nutr. 35: 107.

Fernandez, R. and Phillips, S.F. 1982b. Components of fiber bind iron in vitro. Am. J. Clin. Nutr. 35: 100.

Garcia Lopez, J.S. and Wyatt, J.C. 1982. Effect of fiber in corn tortilla and cooked beans on iron availability. J. Agric. Food Chem. 30: 724.

Kelsay, J.L., Behall, K.M., and Prather, E.S. 1978. Effect of fiber from fruit and vegetables on calcium, magnesium, iron, silicon and vitamin A balances in human subjects. Fed. Proc. 37: 755.

Kelsay, J.L., Behall, K.M., and Prather, E.S. 1979. Effect of fiber from fruit and vegetables on metabolic responses of human subjects. 2. Ca, Mg, Fe and silicon balances. Am. J. Clin. Nutr. 32: 1876.

Layrisse, M., Cook, J.D., Roche, M., Kuhn, N.I., Walker, R.B., and Finch, C.A. 1969. Food iron absorption: A comparison of vegetable and animal foods. Blood 33: 430.

Miller, J. 1979. Ferrous sulfate iron absorption from diets containing wheat flour and shorts by anemic rat. Cereal Chem. 56: 253.

Oberleas, D., Muhner, M.E., and O'dell, B.L. 1966. Dietary metal complexing agents and zinc availability in the rat. J. Nutr. 90: 56.

Reinhold, J.G., Garcia-Lopez, J.S. and Garzon, P. 1981. Binding of iron by fibert of wheat and maize. Am. J. Clin. Nutr. 34: 1384.

Reinhold, J.G. and Garcia Lopez, J.S. 1979. Fiber of the maize tortilla. Am. J. Clin. Nutr. 32: 1326.

Reinhold, J.G., Ismail-Beigi, F., and Faradji, B. 1975. Fiber vs. phytate as determinant of the availability of calcium, zinc and iron of breadstuffs. Nutr. Rep. Int. 12: 75.

Robertson, J.B. and Van Soest, P.J. 1977. Dietary fiber estimation in concentrated feedstuffs. J. Animal Sci. 45, Supp. 1, 254.

Ryan, A.T. Jr., Joiner, L.B., and Ryan, F.B. 1982. "Minitab Reference Manual." The Pennsylvania State Univ., University Park, PA 16802.

Sandstead, H.H., Munos, J.M., Jacob, R.A., Kelsay, L.M., Reck, S.J., Logan, G.M., Dintzis, F.R., Inglett, G.E., and Shuey, W.C. 1978. Influence of dietary fiber on trace element balance. Am. J. Clin. Nutr. 31: S180.

Simpson, K.M., Morris, R.E., and Cook, J.D. 1981. The inhibitory effect of bran on iron absorption in man. Am. J. Clin. Nutr. 34: 1469.

Thompson, S.A. and Weber, C.W. 1982. Copper and zinc binding by dietary fiber sources: An ion exchange column method. J. Food Sci. 47: 125.

Van Soest, P.J. 1973. Collaborative study on acid-detergent fiber and lignin. J. Assoc. Off. Anal. Chem. 56: 781.

Comparison of the Volatile Constituents from Rabbiteye Blueberries (*Vaccinium ashei*) During Ripening

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————— ABSTRACT —————

GLC-MS analyses of steam distillates prepared from Delite, Tifblue, and Woodard cultivars of rabbiteye blueberries at three stages of maturity showed qualitative and quantitative differences in their organic constituents. Fifty-one compounds, consisting of aldehydes, ketones, alcohols, aromatic hydrocarbons, terpenes, esters, and three methyl substituted indanones (the latter tentative) were identified and compared. Most compounds with molecular weights of 100 – 200 amu appeared to increase with maturity and indicate potential use as indices of quality. Terpenes, C_6 unsaturated aldehydes, and unsaturated alcohols were the predominant types of compounds identified in the distillates. Synthetic mixtures of linalool, *trans*-2-hexenal, *trans*-2-hexenol, *cis*-3-hexen-1-ol and geraniol possessed typical fruity aromas reminiscent of fresh blueberry odor-flavor as determined by informal sensory evaluations.

INTRODUCTION

RABBITEYE BLUEBERRIES (*Vaccinium ashei* Reade) have received recent attention in the southeastern United States because of their potential as a cash crop to developing rural areas. Six cultivars have been recently introduced by the Coastal Plain Experiment Station, Tifton, GA (Brightwell, 1969), that are highly prolific, relatively resistant to insects and diseases, and offer high potential for increasing total acreage in production. Increase in production and economic importance of this crop has necessitated definitive analysis of flavor quality, especially characteristic of variety and stages of development.

Previous investigators have identified some volatiles of low-bush blueberries (*Vaccinium angustifolium*) (Hall et al., 1970). Parliment and Kolor (1975) identified 21 compounds in high bush blueberries (*V. corymbosum*) including low molecular weight esters, alcohols, aldehydes, and terpenes. However, only minor attempts were made to relate three of the identified compounds to blueberry aroma. Hirvi and Honkanen (1983) reported identification of 19 compounds. Horvat et al. (1983) described the identification of 42 compounds in Rabbiteye blueberries (*V. ashei*).

This investigation is a continuation of our earlier research on the identification of volatile compounds in Rabbiteye blueberries to establish definitive chemical indices whereby the quality of blueberries may be determined objectively.

MATERIALS & METHODS

SAMPLES of Rabbiteye blueberries (cvs. Delite, Tifblue, and Woodard) at three stages of maturity were obtained from the University of Georgia Horticultural Farm, Athens, GA. The berries were separated into three stages of maturity using the following criteria: trace of anthocyanin pigmentation (pinkish); deep blue coloration but not separating easily from the bush, and deep blue coloration with berries readily released from the bush. Two hundred gram samples of the berries were selected and placed in a Waring Blendor with

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200 mL distilled water; the container was sealed and the berries macerated for 3 min at medium speed. The resulting slurry was placed in a 3-L round-bottom flask containing 400 mL distilled water. A Likens-Nickerson steam distillation, continuous extraction head was attached (Likens and Nickerson, 1964) to the flask and 120 mL of glass distilled pentane was placed in the solvent flask. The isolation was carried out by boiling the pentane and blueberry macerate for 6 hr. After cooling the apparatus, the flask containing the pentane was removed, cooled to ca. 40°C and maintained at this temperature. The extract was then concentrated to ca. 0.5 mL by blowing a gentle stream of high purity nitrogen on its surface and further concentrated to 100 μL by allowing the solvent to evaporate at ambient temperature. Further concentration to 50 μL was later required to identify the trace constituents in these extracts.

GLC-MS analyses

Analyses were performed with a Perkin Elmer Model 900 gas-liquid chromatograph equipped with a flame ionization detector on samples ranging from 0.2 – 1.0 μL in volume. The chromatograph was connected by means of an effluent splitter to a DuPont 21-490B mass spectrometer equipped with differential pumping on the analyzer section. Separations were made on a 50m X 0.05 cm glass open-tubular column coated with OV-1. GLC conditions were: carrier gas inlet pressure, 0.6 kg/cm²; injector and manifold temperature, 250°C; and column programmed from 55°C to 215°C at 1.5°C/min.

Table 1—Volatile compounds of rabbiteye blueberry identified by GLC-MS[a]

methanol	m/e 154; oxygenated terpene (8)
acetaldehyde*	isomer of butylbenzene* (9)
ethanol*	isomer of pentylbenzene
2-pentanone*	linalool* (12)
1-penten-3-ol*	terpinyl acetate[a]
toluene* (1)	isomer of dodecene
hexanal*	geraniol* (13)
2-furfural*	terpinene-4-ol*
trans-2-hexenal (2)	α-terpineol* (14)
trans-2-hexenol	*trans* caran-*cis*-3-ol[a] (15)
cis-3-hexen-1-ol	p-menth-8-ene-10-ol (16)
isomer of xylene	nerol* (17)
isomer of xylene	thymol* (18)
acetylfuran	isomer of octylbenzene[a] (19)
benzaldehyde	carvacrol[b]
pulegone	eugenol* (20)
i-propylbenzene	isomer of divinylbenzene[a] (21)
1,8-cineole	m/e 204; sesquiterpene (22)
myrcene (3)	isomer of pentamethyl-2,3-dihydroindene[a]
allocimene[b]	β-ionone* (23)
isomer of butylbenezene*	2-tridecanone* (24)
terpinolene (4)	m/e 204; sesquiterpene (25)
limonene* (5)	isomer of tetramethylindanone-1[a] (26)
p-cymene* (6)	isomer of tetramethylindanone-1[a] (27)
hexanol* (7)	geranyl formate* (28)
	isomer of pentamethyl-1-indanone[a] (29)
	m/e 204; sesquiterpene
	n-eicosane (31)
	octadecyl alcohol (32)

* Previously identified in Rabbiteye blueberry (*Vaccinium ashei*).
[a] Numbers in parentheses correspond to GLC peaks in chromatograms.
[b] Identified solely on the basis of a comparison of their mass spectra with standards in the literature.

Mass spectrometer conditions were: ion source temperature, 210°C; scan rate, 10 sec per decade; ionizing voltage, 70 eV; and ion source pressure, 2×10^{-5} Torr. Compounds were identified by comparison of their mass spectra and GLC retention times to those of known standards obtained from commercial sources or synthesized by established methods. All reference compounds were analyzed by GLC-MS for purity. Compounds identified on the basis of comparison of their mass spectra with published spectra are designated "tentatively" identified. Estimates of levels of components of interest, *trans*-2-hexenal, *trans*-2-hexenol, linalool and geraniol, were determined by normalization of peak areas. All blueberry isolates were run in duplicate. Precision of the GLC procedure was determined from the results of five repetitive analyses of a standard pentane solution containing these compounds in approximately the same concentration as the isolates.

Odor evaluation of synthetic mixtures

Synthetic mixtures of select compounds identified in this investigation and those identified previously as "impact compounds" (Parliment and Kolar, 1975) were prepared in pentane in ratios similar to that observed in GLC profiles of the volatile fractions of ripe blueberreis. Aliquots of these solutions and the volatile fractions of ripe blueberries were applied to blotter paper, the solvent was evaporated and informal odor evaluations were made by four experienced odor-flavor researchers for their similarity of odor.

RESULTS & DISCUSSION

EACH BLUEBERRY cv. examined showed an increase in medium to high molecular weight compounds with maturity. Fig. 1 shows the GLC chromatograms of the volatile fractions from green, midripe, and ripe Woodard berries. Note that most of the compounds represented by peaks 9 – 36 increased in levels with maturity, whereas peaks 1 through 7 decreased. Similar results were obtained from analysis of the Delite and Tifblue berries.

GLC analysis of the volatile fractions from ripe Woodard, Delite and Tifblue berries revealed qualitative and quantitative differences by cv. (Fig. 2). Highly concentrated samples (50 μL) were required to reveal peaks 2, 8, 16*, 19, 21, 23, 25, 29, 31 through 37 in the Woodard berries
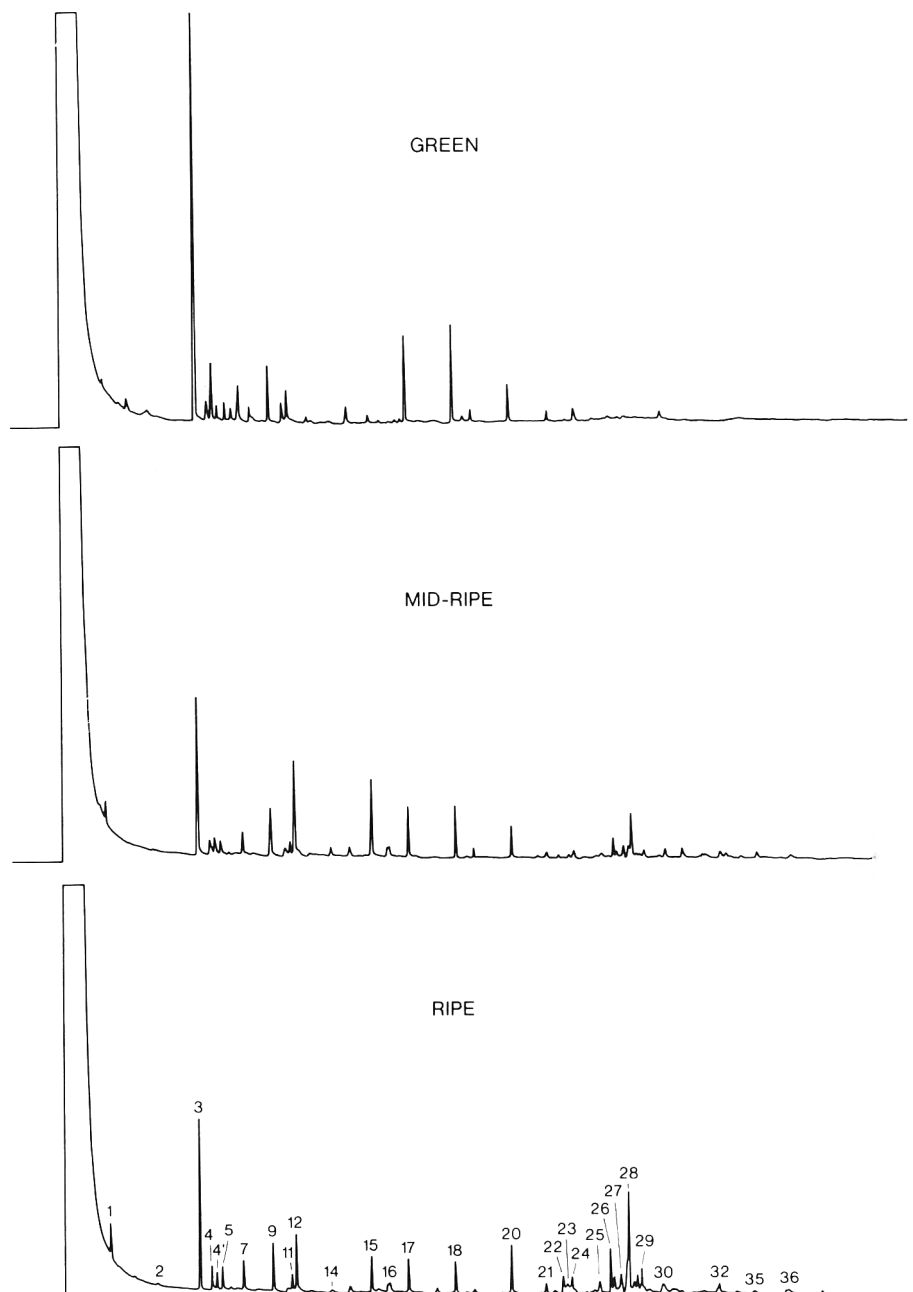

Fig. 1—GLC chromatograms of green (1), midripe (2), and ripe (3) Woodard blueberries.

which were absent in the more dilute concentrates. Analyses of highly concentrated distillates revealed the same range of compounds in all three cvs. Compounds identified are listed in Table 1 in order of elution and include several not identified in our prior reserach (Horvat et al., 1983). Of particular interest are the tentatively identified three methyl-substituted indanones and pentamethyl-2,3-dihydroindene that have not been previously reported in plant materials. The total number of compounds identified from blueberry volatiles (lowbush, highbush, and Rabbiteye) now totals 62. It appears that the range of compounds present in highbush and Rabbiteye blueberries are very similar.

The compounds selected for comparison as indices of maturity in the three ripening Rabbiteye blueberry cvs. were linalool, *trans*-2-hexenal, *trans*-2-hexenol, *cis*-3-hexen-1-ol and geraniol, the latter three having been previously identified as flavor impact compounds by Parliment and Kolor (1975). The importance of *cis*-3-hexen-1-ol and geraniol to blueberry aroma was established in the present study by comparing pentane solutions of these two compounds and the three flavor impact compounds identified by Parliment and Kolor (1975) to ripe blueberry isolates by means of an informal odor panel. The consensus of the panel members was that these synthetic mixtures possessed the same aroma as the blueberry isolates. Estimates of the levels of these impact compounds was determined by GLC. Results of these analyses (Table 2) revealed that *trans*-2-hexenol and *trans*-2-hexenal decreased with ripening in all cvs., whereas linalool reached a maximum in the midripe fruit of two of the cvs. In Tifblue, maximum levels of linalool occurred in the ripe berries (12.9%). However, geraniol reached a maximum concentration in the midripe fruit of all three cvs. Differences in levels of these four compounds in the mature fruit of the three cvs. studied appear to reflect subtle differences that were apparent in aroma and taste, although each cv. possessed a typical blueberry aroma.

Table 2—Concentration of flavor impact components from blueberry volatiles at three stages of maturity

	Delite			Tifblue			Woodard		
	Green	Midripe	Ripe	Green	Midripe	Ripe	Green	Midripe	Ripe
trans-2-hexenal	0.62[a]	0.33[a]	0.34[a]	0.77[a]	0.51[a]	0.46[a]	0.89[a]	0.49[a]	0.47[a]
trans-2-hexenol	0.07	0.05	0.05	0.09	0.04	0.05	0.11	0.08	0.06
cis-3-hexen-1-ol	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.	0.07	0.05	Tr.
linalool	1.02	1.29	1.47	8.70	9.81	12.92	2.65	12.31	8.47
geraniol	2.92	3.40	2.24	0.13	0.28	0.24	0.57	4.08	2.33

[a] Numbers represent percentage of total area of GLC peaks in chromatograms. Relative standard deviation for trans-2-hexenal, trans-2-hexenol, and geraniol was 0.01 and linalool 0.25.
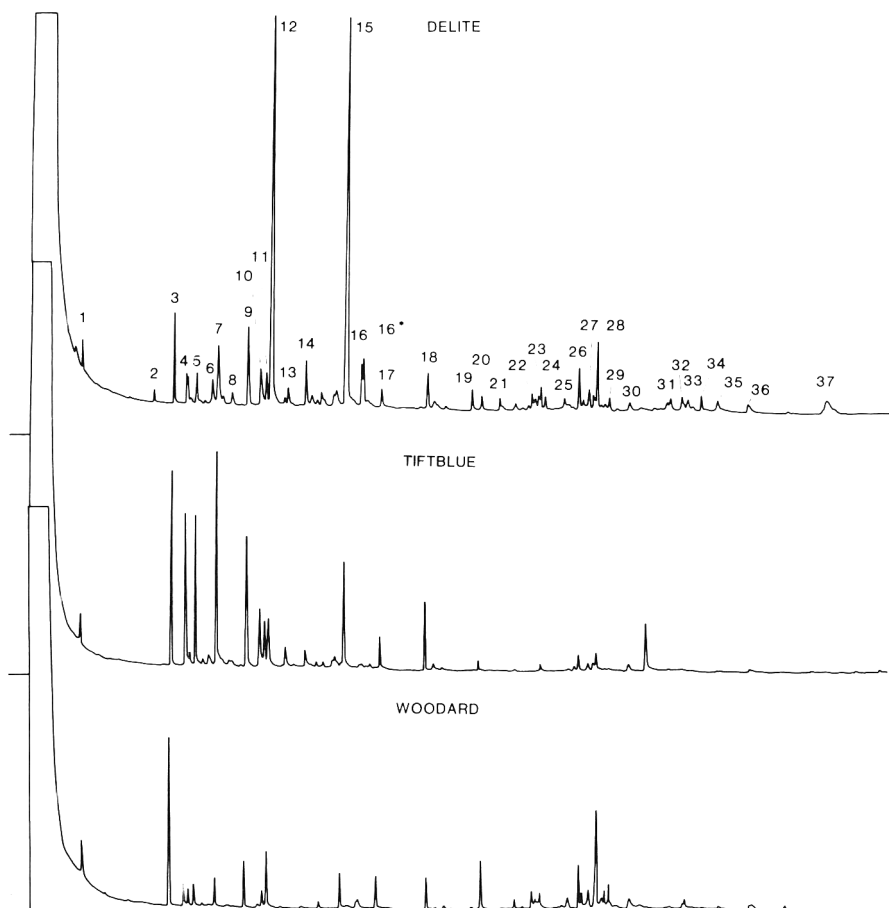

Fig. 2—GLC chromatograms of ripe Delite, Tifblue, and Woodard blueberries.

Leakage of Anthocyanins from Skin of Thawed, Frozen Highbush Blueberries (*Vaccinium corymbosum* L.)

GERALD M. SAPERS, SUSAN B. JONES, and JOHN G. PHILLIPS

——————————ABSTRACT——————————

Factors affecting the tendency of thawed blueberries to leak pigmented exudate were investigated. Drip and anthocyanin leakage rates (ALR) were determined spectrophotometrically. Leakage vs time curves were linear or two-phase linear, ALR varying with cultivar, ripeness, and berry condition. Dewaxing increased ALR with most cultivars. ALR did not correlate with berry anthocyanin content, surface area, or cuticle thickness. ALR and amount of drip were poorly correlated. ALR varied from berry-to-berry within samples. Leakage was observed to be nonuniform on berry surfaces, appearing at skin cracks and ruptures, the calyx area, and other point sources. An hypothesis relating leakage to skin condition, fluid accumulation, and anthocyanin content is presented.

INTRODUCTION

THE LEAKAGE of pigmented exudate through the skin of frozen blueberries during thawing (drip) may detract from product appearance. The extent of drip with frozen strawberries depends on cultivar (Ferry and Cabibel, 1973) and freezing rate (Kaloyereas, 1947). Crivelli and Rosati (1975) reported cultivar differences in drip loss from thawing raspberries and thornless blackberries. Previously, we reported differences in the tendency of highbush blueberries to leak during thawing, Elliott berries being especially prone to this behavior (Sapers et al., 1984b). Differences in SEM images of the epicuticular wax were seen between this cultivar and Burlington, a cultivar not prone to leakage.

With sirup-packed berries, solutes including pigments diffuse from the fruits into the sirup during frozen storage at temperatures above $-18°C$ (Guadagni and Nimms, 1957). Moon et al. (1936) considered the color of frozen blueberries to be enhanced by the pigmented sirup resulting from this process. Guadagni et al. (1960) demonstrated that the diffusion of pigments from frozen raspberries to sirup follows first order kinetics, the rate constant obeying the Arrhenius equation. In a previous study of anthocyanin leakage from raw or cooked highbush blueberries, we obtained linear or two-phase linear leakage vs time curves, leakage rates varying among the cultivars compared and appearing to be associated with the incidence of skin rupturing and with berry pigment content (Sapers et al., 1985). Our objectives in the present study were to determine the mechanism of anthocyanin leakage from thawing blueberries and the basis of cultivar differences in the tendency to leak.

MATERIALS & METHODS

Sample preparation and measurement of leakage rates (dynamic system)

Samples of nine highbush blueberry cultivars (Berkeley, Bluecrop, Bluetta, Burlington, Coville, Earliblue, Elliott, Jersey, and Weymouth) were obtained from the USDA, Rutgers University Blueberry and Cranberry Research Center in Chatsworth, New Jer-

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sey in 1982 (two harvests) and 1983. Cleaned, dry berries were packaged in 1/2-gallon polyethylene freezing containers and frozen at $-13°C$, the containers being lined up in a single row directly facing the blower to assure uniform and rapid freezing. After several days' equilibration, the containers were transferred to the freezer shelves and stored for as long as 18 months.

Anthocyanin leakage was determined with 50g portions of blueberry samples, sorted to exclude atypically large, small, or defective berries and weighed in the frozen state. The berries were thawed overnight at $3°C$ and then equilibrated at $25°C$ for 1 hr. Immediately prior to the determination, drip was removed from the thawed berries by rinsing with three successive 35-mL portions of distilled water. The rinsings were collected under suction, combined and retained for volume measurement and spectrophotometric analysis. After draining and rinsing, selected samples were dewaxed in $CHCl_3$ prior to the leakage measurements. Leakage was determined in a "dynamic" model system by stirring the rinsed or dewaxed, rinsed berries in 500 mL distilled water under standardized conditions: taking aliquots at 4-min intervals over 24 min; filtering the aliquots; diluting the aliquots with pH 3 McIlvaine's buffer; and measuring their absorbance at 519 nm, the absorption maximum of blueberry anthocyanins at this pH, with a Perkin-Elmer Model 552 UV-visible spectrophotometer. Leakage rates were obtained by plotting absorbance vs stirring time curves and measuring their slopes. Procedures for dewaxing and determining leakage rates were described in detail previously (Sapers et al., 1985). The combined rinsings from the thawed berries were diluted with an equal volume of pH 3 buffer and analyzed spectrophotometrically at 519 nm. Data on the condition and number of berries (used to estimate total surface area Sapers et al., 1985) in each portion evaluated for leakage were recorded. Leakage data were compared with values of the total anthocyanin content, titratable acidity, soluble solids content, and soluble solids-acidity ratio (SS/A) determined previously on each berry sample (Sapers et al., 1984a).

Berry-to-berry variation in leakage

To determine whether leakage from thawed samples was subject to berry-to-berry variation, the individual berries in a 50g sample that had been thawed and rinsed by the procedures described above were blotted on absorbent tissue and then distributed in 3 oz plastic cups, each cup containing 20 mL distilled water. After 24 min at approximately 20°C (without stirring), 20 mL pH 3 McIlvaine's buffer was added to each cup, and after mixing, the coloration of the liquid was evaluated subjectively.

To compare potential causes of leakage, sets of five matched berries that were found to be similar in leakage behavior after 15 min in the static system described above, were selected for study. The berries were treated by making a 5-mm slit in the skin with a razor blade, abrading the skin by scraping with the blade perpendicular to the skin, or removing a section of skin with the razor blade. Leakage rates were measured with a scaled-down version of the dynamic system used for 50g samples. Each matched set of berries was added to 100 mL distilled water in a 150 mL beaker and stirred with a 25 mm magnetic stirring bar under standardized conditions for 24 min. Aliquots were taken after 8, 16, and 24 min, diluted with an equal volume of pH 3 buffer, and analyzed spectrophotometrically.

Examination of individual berries

Individual berries from frozen samples were thawed and rinsed as described above. Cross sections of fresh, frozen, and thawed berries were compared visually to determine the extent of anthocyanin diffusion from the skin into the berry interior. After rinsing to remove adhering drip, thawed berries were oriented in different positions and blotted on absorbent tissue to locate sources of leak-

age on the berry surfaces. Leakage sites also were observed with a Bausch and Lomb StereoZoom microscope at a magnification of 14-60X.

Measurement of skin thickness

Cuticle and epidermal cell wall thickness were determined for Bluetta and Elliott blueberries by microscopic examination of embedded sections. The skin specimens (approx 1 mm cubes) were immersion-fixed with 4% glutaraldehyde in 0.01M Na cacodylate pH 7 for 4 hr at room temperature, postfixed with 1% OsO_4 in the same buffer for 2 hr, dehydrated in a graded ethanol series, and embedded in Spurr resin. Following polymerization, sections 1 μm thick were cut with an LKB IV Ultratome and mounted on glass slides, which were later cover-slipped. Measurements of skin thickness were taken with a 16X micrometer eyepiece mounted on an Olympus BH-2 light microscope with a 100X objective. For each cultivar, sections were prepared from five berries, and 40 thickness measurements were made per berry. Skin thickness was operationally defined as the distance from the outer berry surface to the cytoplasmic surface of the epidermal cell outer wall.

Statistical methods

Correlation and regression techniques were used to investigate relationships between leakage rates and other variables. Analysis of variance techniques were used to separate out sources of variability for leakage rates such as cultivar, harvest date and season, and the dewaxing treatment. Comparisons between means were made by application of the Waller-Duncan K-ratio T test (Waller and Duncan, 1969).

RESULTS & DISCUSSION

Anthocyanin leakage from thawed samples

The blueberry samples employed in this study varied in acidity and soluble solids, indicative of possible ripeness differences (Woodruff et al., 1960), as well as in total anthocyanin content and berry surface area, both of which might be expected to influence leakage (Table 1). Differences in composition and surface area between samples harvested in 1982 and 1983 generally were small.

Anthocyanin leakage from thawed blueberries usually could be represented by linear absorbance vs time curves (Fig. 1, see Burlington). Occasionally, samples yielded two-phase linear leakage curves, the second slope being greater than the first (Fig. 1, see Earliblue), indicating the occurrence of an event causing the leakage rate to increase during the trial. On the other hand, many dewaxed samples (Fig. 1, see Bluetta) yielded two-phase linear leakage curves with the second slope smaller than the first, suggesting depletion of the leakage source or an increase in the resistance of the berries to leakage.

Leakage rates (slopes) varied over a 20-fold range (Table 2), depending on cultivar, ripeness, condition, and dewaxing. The highest leakage rates were obtained with samples that underwent extensive skin rupturing during the leakage determination, an indication of poor condition. Comparisons of Berkeley, Bluetta, and Earliblue samples, harvested on two dates during the 1982 season, indicated an effect of berry ripeness on anthocyanin leakage, higher leakage rates being obtained with samples of each cultivar having the higher SS/A ratios (effect significant at 0.01 level by F-test).

Mean anthocyanin leakage rates for 1982 and 1983 samples of nine highbush blueberry cultivars are given in Table 3. The relatively large differences in leakage rates between seasons cannot be explained in terms of differences in sample ripeness (see Table 1). With the exception of the 1982 Earliblue and Weymouth berries discussed previously, all samples appeared to be in good condition, based on the turbidity of the water in which they were stirred. However, the 1982 samples may have been subjected to transient thawing during frozen storage due to unrecorded power outages and/or equipment malfunctions. Such thawing is indicated by visual observation of frozen berry cross sections, showing the occurrence of a narrow zone of pigment diffusion from the skin into the mesocarp. The diffusion zones were wider with 1982 samples than with 1983 samples. One would expect leakage rates to be greater in berries damaged by freeze-thaw cycling. Cultivar differences in leakage rates for samples of similar ripeness and condition, obtained in the same season, were similar in magnitude to ripeness effects on leakage, the rates falling within a three-fold range. Bluecrop and Bluetta berries tended to leak less while Weymouth tended to have a higher leakage rate.

Table 1—Composition and surface area of highbush blueberry samples

Cultivar	Harvest date	Titratable acidity (% citric)	Soluble solids (% at 20°C)	SS/A[a]	Total anthocyanin[b]	Total surface area (cm²)[c]
Berkeley	7-22-82	0.53	13.8	26.0	154	206
	8-2-82	0.40	13.6	33.6	148	185
	7-19-83	0.36	12.4	35.1	102	173
Bluecrop	7-16-82	0.56	11.9	21.4	86	177
	7-23-82	0.64	12.5	19.6	84	175
	7-19-83	0.65	13.6	21.0	66	185
Bluetta	6-28-82	0.72	10.9	15.3	168	201
	7-9-82	0.48	12.2	25.8	154	211
	7-5-83	0.50	11.1	22.4	135	198
Burlington	8-2-82	0.70	14.8	21.5	270	223
	8-2-83	0.98	13.2	13.5	152	235
Coville	8-2-83	0.65	14.1	22.0	143	192
Earliblue	6-28-82	0.50	12.5	25.0	140	196
	7-2-82	0.34	12.2	36.3	135	193
	7-5-83	0.31	14.0	44.8	153	194
Elliott	8-3-82	1.26	11.3	9.0	224	204
	8-9-82	1.36	11.5	8.4	204	207
	8-2-83	1.37	16.2	11.9	233	227
Jersey	7-19-83	0.71	14.8	20.9	164	224
Weymouth	6-28-82	0.54	11.4	21.0	152	204
	7-6-82	0.45	11.0	24.4	132	207
	7-5-83	0.84	11.2	13.2	129	217

[a] Soluble solids ÷ titratable acidity
[b] Absorbance of ethanolic extract at 543 nm X dilution factor
[c] For 50g sample

The highest leakage rates obtained with thawed berries were substantially smaller than rates measured previously with coooked samples (Sapers et al., 1985).

Dewaxing increased the leakage rate with Bluetta, Burlington, Coville, Elliott, and Jersey blueberries, presumably by removing a barrier to the diffusion of polar anthocyanins from the underlying epidermal cells. It is not clear why the other cultivars did not respond similarly. However, their behavior was consistent over two seasons. Norris (1974) observed an increase in the penetration of 2,4-D through isolated plant cuticles after dewaxing. The removal of wax from blueberry skin also might affect its mechanical properties, making it more vulnerable to cracks and tears that would represent potential leakage sites.

The linear relationship found between absorbance and stirring time suggests that the leakage process may be described by Fick's law of diffusion, according to which the anthocyanin diffusion rate across the blueberry cuticular membrane should be directly proportional to the berry surface area and anthocyanin concentration gradient and inversely proportional to the membrane thickness. Our data were not consistent with this hypothesis, however; correlations between leakage rates and the sample anthocyanin content (assumed to be proportional to the anthocyanin concentration in epidermal cells), the sample surface area, or the product of the anthocyanin content and surface area lacking significance except with the dewaxed 1983 berries (Table 4). Although not significant at the 5% level, correlation coefficients for the 1982 samples also were higher for dewaxed berries than for corresponding untreated samples. The lack of significance in 1982 may be a reflection of the smaller number of cultivars compared, data for Earliblue and Weymouth samples being excluded because of atypical berry condition.

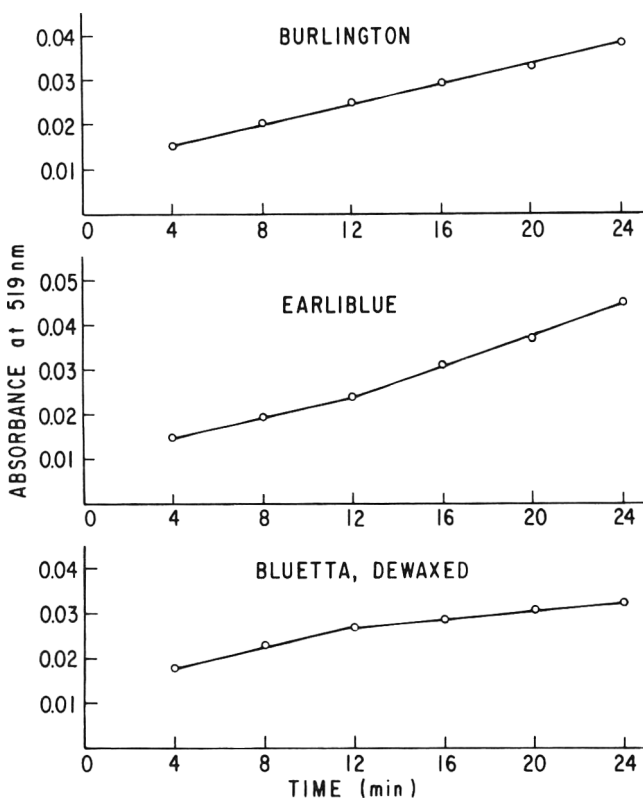Leakage rates for 1983 Elliott and Bluetta samples, untreated or dewaxed (see Table 3), were compared with

measured cuticle and epidermal cell wall thicknesses (1.8 and 3.6 μm for Elliott, and 1.2 and 3.0 μm for Bluetta, respectively) to determine conformity to Fick's law. Leakage rates were not inversely proportional to the thickness of either or both structures, as would be expected if Fick's law were operative, even when rates were corrected for differences in berry anthocyanin content and surface area. Norris (1974) found no correlation between the leaf or fruit cuticle thickness of various plants and the penetration of 2,4-D. Likewise, Schönherr (1976) reported that permeability coefficients for the diffusion of tritiated water across cuticles isolated from the leaves of several plants were not inversely proportional to cuticle thickness.

These results indicate that anthocyanin leakage is a more complex phenomenon than simple diffusion through the cuticle. Apparently, the cuticle per se is not an effective barrier to diffusion. The epicuticular wax is an effective barrier, and its removal by dewaxing in chloroform makes the leakage process appear more like diffusion through a membrane, perhaps the semipermeable plasma membrane of epidermal cells. With berries that are not dewaxed, leakage rates may be limited by diffusion through more permeable sites or discontinuities in the epicuticular wax.

Correlation between leakage rate and drip

Although the thawed berry samples evaluated in this study were visibly wet, they did not leak sufficient exudate during thawing to produce a measureable drip volume. Therefore, the extent of drip loss was estimated by rinsing the thawed berries with water and measuring the total quantity of anthocyanin in the rinsings (Table 3). In prin-

Fig. 1—Anthocyanin leakage curves for thawed highbush blueberries (1983 season).

Table 2—Effect of ripeness and condition on anthocyanin leakage from thawed blueberries—1982 season

Cultivar	Harvest date	SS/A[a]	Condition	Leakage rate ($\times 10^{-3}$)[b]	
				Not dewaxed	Dewaxed
Berkeley	7-22-82	26.0	Good	2.2	3.3
	8-2-82	33.6	Good	4.9	3.7
Bluetta	6-28-82	15.3	Good	1.8	4.0
	7-9-82	25.8	Good	4.0	—
Earliblue	6-28-82	25.0	Fair	5.3	8.1
	7-2-82	36.3	Poor	16.7	13.1
Weymouth	6-28-82	21.0	Fair	8.9	8.5
	7-6-82	24.4	Poor	42.6	22.1

[a] Soluble solids (%) ÷ titratable acidity (% citric)
[b] Absorbance units per min per 100g berries

Table 3—Cultivar differences in anthocyanin leakage from thawed blueberries

Cultivar	Leakage rate ($\times 10^{-3}$)[a]				Drip (1983)[b]
	1982		1983		
	Not dewaxed	Dewaxed	Not dewaxed	Dewaxed	
Berkeley	3.6[ef]	3.5[g]	2.1[cd]	2.5[ef]	42.2[d]
Bluecrop	3.1[ef]	3.2[g]	0.7[e]	0.7[g]	3.0[g]
Bluetta	2.8[f]	4.0[fg]	0.8[e]	2.1[f]	14.0[f]
Burlington	4.0[ef]	4.9[f]	2.1[cd]	3.4[d]	50.2[c]
Coville	—	—	1.9[d]	2.9[de]	18.2[f]
Earliblue	11.0[d]	10.6[d]	1.9[d]	2.4[ef]	18.3[f]
Elliott	4.6[e]	8.7[e]	1.7[d]	4.1[c]	37.8[de]
Jersey	—	—	2.1[cd]	3.3[d]	15.6[f]
Weymouth	20.6[c]	15.3[c]	2.6[c]	2.3[ef]	37.4[e]

[a] Absorbance units per min per 100g berries
[b] Absorbance units per 100g berries
[c-g] Means with different superscripts in same column are significantly different (p < 0.05)

ciple, this estimate of drip should be related to the leakage rate, as determined in the dynamic system, since drip represents leakage over an extended period of time. However, the correlation between these two measurements, while significant, was too low ($r = 0.57$) to be of any predictive value.

A number of factors can explain the poor correlation between drip and leakage rates. First, drip represents a slow process occurring over a wide temperature range while leakage rates are measured over a brief time interval at a constant and higher temperature. Second, the drip process differs qualitatively from leakage (as we measured it), resulting from the slow melting of ice crystals in the intercellular spaces, diffusion of pigments and other solutes from damaged epidermal cells, and the dilution of these cellular fluids by the melted ice. The quantity of intercellular ice and extent of cell damage depend on freezing rate and storage conditions (Joslyn, 1966). During thawing, the pigmented cellular fluids accumulate within the berries and also penetrate the skin as exudate. Only this last step is measured as leakage in our system. In spite of the poor correlation between drip and leakage. We believe that both measurements are useful in understanding the basis of processability and the effects of cultivar and sample condition on processability.

Anthocyanin leakage from individual berries

Observations made with individual thawed berries in the static model system shed considerable light on the leakage process. Of primary importance is the fact that leakage within a berry sample is not uniform but varies greatly from berry-to-berry, some berries even exhibiting no leakage (Table 5). The percentages of nonleaking and leaking berries were not highly correlated with leakage rates for 50g samples, probably because of the large contribution to anthocyanin leakage made by a relatively small number of berries. Nevertheless, clear differences in the distribution of leaking berries can be seen with widely differing samples such as Burlington and Weymouth. With the latter cultivar, the effects of berry condition on the leakage rate and distribution are evident. Similarly, dewaxing increases the proportion of leaking berries as well as the leakage rate.

Close examination of leaking berries revealed an association between the severity of leakage and the occurrence of skin rupturing that resulted in the release of seeds and other particulate matter as well as anthocyanin. However, not all anthocyanin leakage was accompanied by turbidity or visible skin rupturing. To determine the effects of skin abrasion, rupturing, and fragmentation on anthocyanin leakage rates, these defects were simulated in sets of berries matched according to the extent of leakage in the static system by scraping, slitting, or removing a section of skin on each berry. Leakage rates determined for these sets with the scaled down dynamic model system are given in Table 6. As might be expected, leakage rates for berries classified in the "slight leakage" category were higher than rates for berries classified as nonleaker. Leakage rates for the latter were not increased by abrading the skin with a razor blade. However, slitting the skin did increase the leakage rate by permitting the escape of pigmented cellular fluids released from cells that had collapsed during thawing. Skin fragments trimmed from the berries did not release much anthocyanin in spite of their high pigment content. However, the berries from which skin fragments were taken gave relatively high leakage rates. It is likely

Table 4—Correlations between anthocyanin leakage rates and total anthocyanin content and surface area for untreated and dewaxed blueberry samples

	Correlation Coefficient	
	1982[a]	1983[b]
Untreated		
Leakage rate vs total anthocyanin	0.41	0.30
Leakage rate vs surface area	0.16	0.29
Leakage rate vs total anthocyanin X surface area	0.37	0.30
Dewaxed		
Leakage rate vs total anthocyanin	0.63	0.90[c]
Leakage rate vs surface area	0.46	0.71[d]
Leakage rate vs total anthocyanin X surface area	0.59	0.90[c]

[a] Five cultivars compared
[b] Nine cultivars compared
[c] Significant at $p = 0.01$
[d] Significant at $p = 0.05$

Table 6—Anthocyanin leakage from simulated defects in skin of Berkeley blueberries[a]

Trial	Appearance in static system	Treatment	Leakage rate $(\times 10^{-3})$[b]
1	No leakage	None	2.4
	No leakage	Skin abraded	1.5
	Slight leakage	None	8.1
2	No leakage	None	1.2
	No leakage	Skin slit	3.5
	No leakage	Skin fragments	1.6
3	No leakage	None	2.8
	No leakage	Skin fragments	4.1
	No leakage	Berry after skin fragments removed	7.6

[a] Harvested 8/2/82
[b] Slope of absorbance vs time curve (absorbance units per min per 100g berries)

Table 5—Anthocyanin leakage from individual berries

Sample[a]	Treatment	Condition	Leakage rate $(\times 10^{-3})$[b]	Percentage of berries		
				No leakage	Slight leakage[c]	Moderate leakage[c]
Burlington 8/2	None	Good	4.0	87	13	0
Bluetta 6/28	None	Good	1.8	67	25	8
	Dewaxed	Good	4.0	26	44	30
Elliott 8/3	None	Good	5.0	38	47	15
	Dewaxed	Good	12.6/8.4[d]	9	59	31
Weymouth 6/28	None	Fair	8.9	64	21	14
7/6	None	Poor	42.6/29.8[d]	22	44	34

[a] 1982 season
[b] Slope of absorbance vs time curve (absorbance units per min per 100g berries)
[c] Slight = trace of pink color; moderate = light pink.
[d] Two-phase linear curve with rates corresponding to slope 1/slope 2

that this leakage represents anthocyanin that diffused from epidermal cells within the skin into the mesocarp during thawing and was released along with accumulating cellular fluids when the barrier represented by the skin was removed. Examinations of cross sections of fresh, frozen, and thawed blueberries clearly show the formation of a pigment gradient from the skin to the interior during thawing.

To determine the site of leakage in berries not having visible skin defects, the surfaces of individual berries were examined by the blotting technique and by microscopic observation. Leakage appeared to come from scattered point sources on the berry surface rather than from the entire surface as would be the case if anthocyanin diffusion from epidermal cells through the cuticle were uniform. Under the microscope, the point sources of leakage could be seen as small droplets of juice flowing from cracks or punctures in the skin. Leakage also appeared at the blossom end of the berries, apparently coming from the calyx. No leakage was seen at the stem scar.

These results suggest that differences in leakage between samples may be due at least in part to differences in the mechanical strength, thickness, and/or condition of the skin which affect the development of point source leaks. Additional differences between samples in the accumulation of cellular fluids under the skin, due to tissue damage during freezing and storage, may affect the pressure exerted by such fluids. Leakage will occur when the fluid pressure is sufficient to break through weak points in the skin. According to our hypothesis then, sample leakage rates reflect the number of point source leaks (a characteristic of the berry skin), the volume leaked (a manifestation of tissue damage and ice formation), and the anthocyanin concentration of the leaking fluid (determined by cultivar and ripeness). Linearity implies a constant flow of exudate from multiple point source leaks. Two-phase linear leakage curves where the second slope is greater than the first slope may represent the occurrence of a major new point source leak (i.e., an extensive skin rupture) during the period of measurement. Leakage curves where the second slope is less than the first may result from the cessation of one or several major point source leak due to decreasing internal pressure. Research to confirm this hypothesis and examine its implications is continuing.

REFERENCES

Crivelli, G. and Rosati, P. 1975. Researches on suitability of raspberry and blackberry varieties to quick freezing. Annali dell Istituto Sperimentale per la valorizzazione tecnologica dei prodotti agricoli 6: 61.
Ferry, P. and Cabibel, M. 1973. Some characteristics of exudation and the exudate from frozen strawberries. Revue Generale du Froid 64(5): 473. (FSTA 074613 74-01-J0031).
Guadagni, D.G., Kelly, S.H., and Ingraham, L.L. 1960. Kinetics of color, ascorbic acid, and total acid diffusion in frozen sirup-packed raspberries. Food Res. 25: 464.
Guadagni, D.G. and Nimms, C.C. 1957. Time-temperature tolerance of frozen foods. 9. Effect of time and temperature on color distribution in retail packs of frozen raspberries. Food Technol. 11: 604.
Joslyn, M.A. 1966. The freezing of fruits and vegetables. In "Cryobiology," Ch. 12, (Ed.) H.T. Meryman. Academic Press, New York.
Kaloyereas, S.A. 1947. Drip as a constant for quality control of frozen foods. Food Res. 12: 419.
Moon, H.H., Smart, H.F., and Caldwell, J.S. 1936. Varietal suitability of cultivated highbush blueberries for freezing in consumer packages. Fruit Products J. 15: 229.
Norris, R.F. 1974. Penetration of 2,4-D in relation to cuticle thickness. Amer. J. Bot. 61: 74.
Sapers, G.M., Burgher, A.M., Phillips, J.G., and Jones, S.B. 1984a. Color and composition of highbush blueberry cultivars. J. Amer. Soc. Hort. Sci. 109(1): 105.
Sapers, G.M., Burgher, A.M., Phillips, J.G., and Jones, S.B. 1984b. Effects of freezing, thawing, and cooking on the appearance of highbush blueberries. J. Amer. Soc. Hort. Sci. 109(1): 112.
Sapers, G.M. and Phillips, J.G. 1985. Leakage of anthocyanins from skin of raw and cooked highbush blueberries. J. Food Sci. 50: 437.
Schönherr, J. 1976. Water permeability of isolated cuticular membranes: The effect of cuticular waxes on diffusion of water. Planta (Berl.) 131: 159.
Waller, R.A. and Duncan, D.B. 1969. A Bayes rule for the symmetrical multiple comparisons problem. J. Amer. Statistical Assoc. 64: 1484.
Woodruff, R.E., Dewey, D.H., and Sell, H.M. 1960. Chemical changes of Jersey and Rubel blueberry fruit associated with ripening and deterioration. Proc. Amer. Soc. Hort. Sci. 75: 387.
Ms received 6/15/84; revised 9/14/84; accepted 10/4/84.

CONCLUSION

THE ODOR-FLAVOR characteristics of Rabbiteye blueberries is a complex mixture of organic compounds in which 51 have been identified in this study. In addition to the compounds previously related to flavor impact in blueberry aroma, two additional compounds, *cis*-3-hexen-1-ol and geraniol, were related to the aroma profile of this commodity. Synthetic mixtures of these compounds and those previously identified as being significant to blueberry aroma possessed typical aroma profiles as determined by informal sensory evaluations.

REFERENCES

Brightwell, W.T. 1969. Rabbiteye blueberries. Ga. Agr. Expt. Sta. Res. Bull. 100: 1.
Hall, I.V., Forsyth, F.R., and Lightfoot, H.J. 1970. Volatiles from developing fruit of Vaccinium angustifolium. Can. Inst. Food Technol. 3: 1.
Hirvi, T. and Honkanen, E. 1983. Application of mass fragmentographic SIM techniques to the analysis of volatile compounds of berries, especially of th genera Vaccinium and Fragaria, in "Instrumental Analysis of Foods," (Ed.) Charalambous, G. and Inlett, G. Academic Press, New York.
Horvat, R.J., Senter, S.D., and Dekazos, E.D. 1983. GLC-MS analysis of volatile constituents in rabbiteye blueberries. J. Food Sci. 48: 278.
Likens, S.T. and Nickerson, G.F. 1984. Detection of certain hop oil constituents in brewing products. Am. Soc. Brew. Chem. Proc. 5.
Parliment, T.H. and Kolar, M.G. 1975. Identification of the major volatile components of blueberry. J. Food Sci. 49: 762.
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Leakage of Anthocyanins from Skin of Raw and Cooked Highbush Blueberries (*Vaccinium corymbosum* L.)

GERALD M. SAPERS and JOHN G. PHILLIPS

―――――――――ABSTRACT―――――――――

Anthocyanin leakage from raw, dewaxed, or cooked blueberries was determined by spectrophotometric analysis of water in which berries were stirred. Leakage did not occur with fresh berries but was observed in samples refrigerated for 5 wk that contained soft berries. Dewaxing produced minimal leakage except with samples of poor condition. Pigment losses from dewaxed berries probably resulted from rupturing of the weakened skin. Leakage from cooked berries was more extensive, leakage vs stirring time curves being linear or two-phase linear. Leakage rates for cooked berries varied among the cultivars compared and appeared to be associated with the incidence of skin rupturing and with berry pigment content.

INTRODUCTION

THE LEAKAGE OF EXUDATE from the skins of fruits greatly influences product appearance and acceptability, especially if the exudate is pigmented. Dekazos and Smit (1976) attributed leakage of juice from fresh rabbiteye blueberries, a storage defect, to physiological breakdown, resulting from changes in the berry environment within closed containers. The loss of cellular fluids from thawing plant tissue (drip) depends on the method of freezing, the conditions and duration of frozen storage, the method of thawing, and the measurement technique (Joslyn, 1966). Previously, we observed cultivar-related differences in the tendency of highbush blueberries to leak during thawing and to impart color to water during cooking (Sapers et al., 1984b).

These phenomena are related to the integrity or permeability of the skin to cellular fluids and can be understood in terms of the barrier properties of the different structures comprising the skin, i.e., the outermost epicuticular wax layer, the underlying cuticle, and the epidermal and subepidermal cell layers in which the anthocyanin pigments are located. Schönherr (1976) found that the water permeability of isolated cuticular membranes was completely determined by the cuticular wax and was independent of membrane thickness. Norris (1974) reported that dewaxing greatly increased the permeability of isolated plant cuticles to 2,4-dichlorophenoxyacetic acid (2,4-D). Albrigo et al. (1980) observed that weight loss due to the dehydration of wild blueberries (*Vaccinium elliotti* Chapm.) was usually greater with black-colored berries than with blue-colored berries which have a higher wax content.

Our objective in this study was to relate anthocyanin leakage from the epidermal cells of blueberries during refrigerated storage and cooking to the barrier properties of the skin and its constituent parts in order to understand the mechanism of leakage and the physical basis of cultivar differences in leakage behavior. Leakage during thawing of frozen blueberries will be the subject of a separate report.

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MATERIALS & METHODS

Source and treatment of blueberries

Samples of seven highbush blueberry cultivars (Berkeley, Bluecrop, Burlington, Coville, Elliott, Jersey, and Weymouth) were obtained from the USDA, Rutgers University Blueberry and Cranberry Research Center in Chatsworth, NJ, in 1983. Cleaned, dry berries were packaged in 1/2-gallon polyethylene containers, covered with lids having 4 mm diam. holes at 3 – 4 cm intervals to permit gas exchange, and stored at 3°C for as long as 5 wk.

Anthocyanin leakage was determined with 50g portions of berries that had been sorted to exclude atypically large, small, or defective berries and then equilibrated at about 25°C for 1 hr prior to the determination. To determine the effects of cooking on anthocyanin leakage, 50g portions of refrigerated berries were placed in a small wire kitchen strainer, held over rapidly boiling water in a stainless steel beaker heated on a hot plate (covered with a metal lid), and steamed for exactly 1 min under standardized conditions. The steamed berries were cooled by immersion in ice water for 1 min and then rinsed with three successive 30 – 35 mL portions of distilled water in a 60 mL coarse porosity fritted glass filter funnel under suction to remove residual cooling water prior to the measurement of leakage.

To determine the effect of epicuticular wax on anthocyanin leakage, 50g portions of fresh berries, equilibrated to 25°C, were dewaxed by stirring for 1 min in 100 mL $CHCl_3$ under standardized conditions at ambient temperature. Dewaxed berries were separated from the $CHCL_3$ in a fitted glass filter funnel under sucion.

To determine the effect of berry condition on anthocyanin leakage, refrigerated samples were sorted prior to weighing to exclude berries with visible defects. Berries that were soft to touch but appeared sound in other respects were either included or rejected, depending on the purpose of the experiment.

Model system for evaluation of anthocyanin leakage

Following equilibrium to room temperature (and rinsing of steamed berries), each preweighed portion of berries was added to 500 mL distilled water in a 600 mL beaker containing a 50 mm magnetic stirring bar and was stirred under standardized conditions for 24 min. At 4 min intervals, approximately 10 mL aliquots were withdrawn with a wide tip serological pipet and filtered through Whatman No. 1 paper under suction to remove seeds, wax and other particulate matter released from the stirring berries. A 5 mL portion of filtrate was added to 5 mL pH 3 McIlvaine's buffer (Hodgman, 1954), and the remaining filtrate was returned to the sample. Following the 24 min stirring procedure, the number and condition of berries in the samples and the turbidity of the water were noted.

Anthocyanins in the diluted aliquots were estimated spectrophotometrically at 519 nm, the visible absorption maximum, with a Perkin-Elmer Model 552 UV-visible spectrophotometer. Since anthocyanin leakage usually was linear or two-phase linear with time, the rate of leakage was determined as the slope of the absorbance vs time curve, calculated by linear regression analysis. Leakage rates were determined with 4 – 5 replications.

Leakage rates were compared with berry total anthocyanin content, determined by spectrophotometric analysis of acidified ethanolic extracts of blueberry samples (Sapers et al., 1983); the soluble solids-titratable acidity ratio (SS/A) (Sapers et al., 1984a); and total surface area, estimated from the weight (W) and number of berries (N) in a sample, assuming the berries to be spherical and to have a density of 1.04 (corresponding to a 10% sugar solution): Total surface area (cm^2) = 4.71 $(W^2N)^{1/3}$.

Statistical methods

Comparisons of leakage rates between cultivars were made by an analysis of variance and application of Scheffe's test (Scheffe, 1953). Relationships between the leakage rates and various other parameters were investigated by means of correlation analysis.

RESULTS & DISCUSSION

Anthocyanin leakage from raw and dewaxed blueberries

Raw highbush blueberries from fresh samples showed no significant anthocyanin leakage when tested in our model
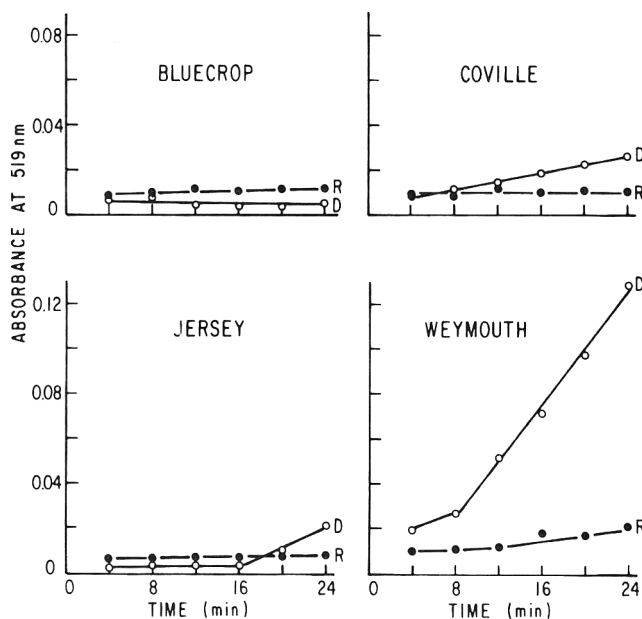

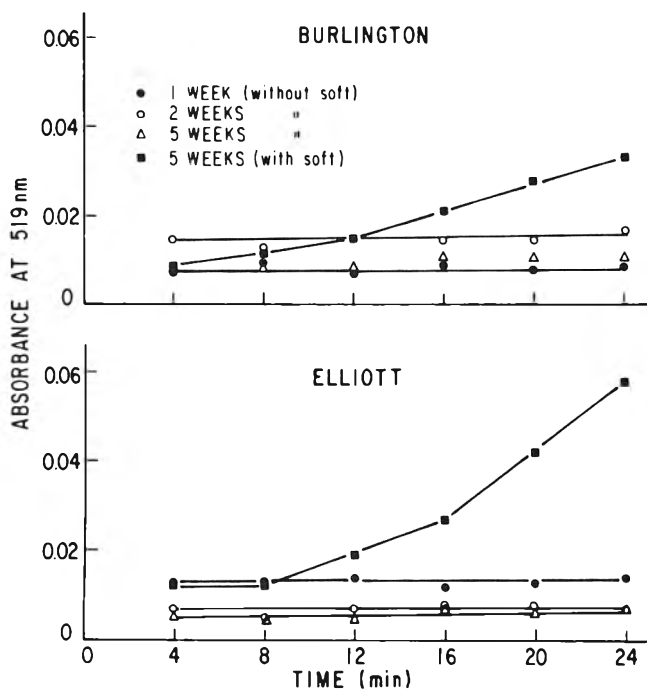*Fig. 1—Anthocyanin leakage from raw (R) and dewaxed (D) blueberries.*

system, as would be expected from common experience (Fig. 1, Bluecrop, Coville and Jersey). Raw Weymouth berries showed slight anthocyanin leakage and also released some particulate matter during stirring, behavior not shown by the other samples and an indication that this sample was in poor condition. The sample apparently was not over-ripe, in fact having a low SS/A value (13.2) indicative of an early stage of ripeness (Woodruff et al., 1960).

Dewaxed blueberries behaved similarly, most samples releasing little or no anthocyanin (Fig. 1, Bluecrop). However, in some cases, minor leakage occurred, usually accompanied by turbidity. Absorbance vs time curves appeared to be linear (Fig. 1, Coville), sometimes preceded by a time interval during which leakage was minimal or absent (Fig. 1, Jersey). The latter behavior suggests the occurrence of a single event causing leakage such as the sudden puncturing or rupturing of the skin of one berry. Visible skin splitting was seen with dewaxed Weymouth berries, the more extensive anthocyanin leakage found with this sample being observed primarily in the vicinity of the skin ruptures. These results clearly show that removal of the epicuticular wax *per se* does not greatly affect anthocyanin leakage from raw berries, other barriers in the skin limiting the leakage rate. However, dewaxing may weaken the berry cuticle sufficiently to allow the skin to rupture, permitting some leakage from the exposed edges or undersurface of the torn skin.

Anthocyanin leakage from stored blueberries

If blueberries are refrigerated for a prolonged period of time, some softening and decay will occur (Dekazos and Smit, 1976). When we evaluated sound, firm berries stored for as long as 5 wk (soft berries being removed from samples during sorting), we measured no anthocyanin leakage (Fig. 2), although the water in which the berries were stirred usually became turbid. However, with samples containing soft berries (not removed during sorting), significant anthocyanin leakage did occur. Leakage was accompanied by skin rupturing in as many as 10% of the berries being tested, a proportion similar to the percentage of soft berries in the sample (9% for Burlington and 15% for Elliott). Thus, anthocyanin leakage from stored blueberries depends on the extent to which individual berries undergo softening,
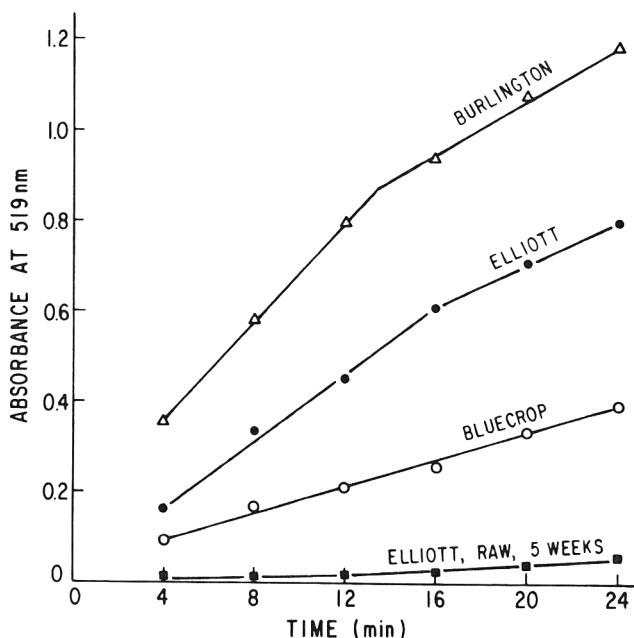

Fig. 2—Anthocyanin leakage from raw Burlington and Elliott blueberries after refrigerated storage.

Fig. 3—Anthocyanin leakage from fresh, steam-cooked blueberries.

Rheological Behavior of Soya Oil-Water Emulsions: Dependence upon Oil Concentration

N. GLADWELL, M. J. GRIMSON, R. R. RAHALKAR, and P. RICHMOND

─────────── ABSTRACT ───────────

Stress-strain measurements have been performed in the linear region for soya oil-water emulsions stabilized with xanthan gum for various oil concentrations and for xanthan gum solutions for various xanthan gum concentrations. The big increase observed in the shear modulus for the emulsions (as compared to the solutions) is primarily due to the interaction between xanthan gum molecules and emulsion droplets. The results obtained are in broad agreement with the existing theories for concentrated dispersions and tend to show that the interdroplet interaction is similar to that expected for 'soft' deformabe spheres. The results for xanthan gum solutions can be explained if the xanthan gum solutions are not true solutions, but some form of dispersions.

INTRODUCTION

OIL-WATER EMULSIONS form the basis of many food systems. The disperse phase concentration ranges from a few percent (e.g. orange squash) to 80% (e.g. mayonnaise). Many food emulsions are viscoelastic. When a small shear stress is applied, the emulsion behaves like a solid by storing most of the energy. At high stress values, the emulsion behaves like a liquid in that the flow occurs. For intermediate values of shear stress, some of the energy is stored in the system while the rest is dissipated, which is characteristic of a viscoelastic fluid.

Shear modulus is a measure of the elasticity of the emulsion. The higher the shear modulus, the greater is the capacity of the emulsion to store energy. In practical terms, it means that a certain shear stress has to be applied before the flow can occur. A high value of shear modulus is indicative of enhanced emulsion stability in low stress situations, e.g. during storage and transportation. On the other hand, it also means that higher stresses (and hence greater pressure drops) are required before the emulsion can flow, resulting in greater pumping and processing costs. High shear modulus usually implies high viscosity which also increases pumping and handling difficulties.

Shear modulus is dependent upon several emulsion parameters, such as, the disperse phase concentration, droplet diameter, polydispersity etc. (Dickinson and Stainsby, 1982). It is also possible to link shear modulus with colloidal interation between the droplets (Buscall et al., 1982). To measure shear modulus of the emulsions, it is necessary to carry out the experiments in the linear region, where there is no breakdown of emulsion structure. At high deformation values, breakdown of the coagulation structure takes place, resulting in liquid-like flow. Shear modulus of suspensions have been reported by serveral investigators. Zosel (1982) and Buscall et al. (1982) carried out the shear modulus measurements on polystyrene latex suspension. Strong dependence upon polystyrene concentration was observed in both cases. The dependence of shear modulus

upon droplet size was studied by Sherman (1970). The shear modulus was found to decrease with increasing drop volume. The objective of this study to investigate the relationship between colloidal interactions and bulk rheology. We have investigated the dependence of shear modulus upon the oil content over a wide range of concentrations. The emulsions were stabilized by xanthan gum. As a comparison, variation of shear modulus with xanthan gum concentration was also studied for xanthan solutions.

MATERIALS & METHODS

DEIONIZED WATER was used for the preparation of emulsions. A soyabean oil with fatty acid composition 16:0 9.8%, 18:0 3.9%, 18:1 20.1%, 18:2 51%, 18:3 7.1% was used. Emulsifer was egg yolk powder and the stabilizer was xantaan gum, both of which were supplied by Colman's of Norwich.

Emulsions were prepared with oil concentration ranging from 20% (comparable to salad dressings) to 70% (comparable to mayonnaise) while keeping the amount of egg yolk and xanthan gum constant. Table 1 lists the composition of A series emulsions and corresponding xanthan gum solutions (assuming all the xanthan gum in the emulsions was in the aqueous phase). B series of emulsions, with composition identical to the A series but with no xanthan gum was also prepared. Further details of materials and the method of emulsion preparation are given elsewhere (Hennock et al., 1984).

Particle size measurements

Average droplet sizes of the emulsions were measured by using a Malvern particle size analyser Model 2600 MSD. The technique works essentially by measuring the diffraction pattern of the emulsion as a monochromatic beam of laser light (red light $\lambda = 633$ nm) is passed through it. The details of the technique are given elsewhere (Hennock et al., 1984).

Rheology

The creep recovery measurements were carried out using a series II Deer rheometer. A concentric cylinder assembly, with inner and outer diameters 12 mm and 15 mm, respectively, was employed. The inner cylinder could be subjected to very small angular displacements (typically a fraction of a degree) by applying a fixed amount of torque. The strain slowly creeps up to the equilibrium value. The stress is then removed and the strain decay is observed, the two responses together giving the creep/recovery behaviour. The calculations of shear stress and shear strain were made by standard procedures (Whorlow, 1980).

RESULTS & DISCUSSION

FIG. 1 shows a typical creep-recovery response of the emulsions. The response essentially consists of two parts. When a fixed stress was applied, the elastic compliance rose instantaneously to a value Jo, equal to *reciprocal of the* instantaneous elastic modulus. After that, there was a time dependent build up which consists of the equilibrium compliance and the viscous compliance. The experiment was repeated for several different values of stress. The instantaneous strain (γ), associated with Jo (= γ/σ) was plotted

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a consequence of "physiological breakdown" (Woodruff et al., 1960) and/or bruising (Ballinger et al., 1973) and not a generalized deterioration in the barrier properties of the skin affecting all berries.

Anthocyanin leakage from cooked fresh blueberries

Anthocyanin leakage from steam-cooked fresh blueberries greatly exceeded that from raw berries, even deteriorated berries stored 5 wk (Fig. 3). Absorbance vs time curves for the five cultivars compared usually were two-phase linear, the second slope being smaller than the first (see Burlington and Elliott). Occasional trials yielded linear or curvilinear leakage curves (see Bluecrop). Leakage rates (slopes 1 and 2, corresponding to each portion of the two-phase linear curves) varied greatly among replicates of the same sample and appeared to be related to the occurrence of berries having visible skin ruptures after 24 min stirring (Table 1). Correlation coefficients for slope 1 vs % ruptures were 0.94 and 0.78 for Elliott and Jersey, respectively). Leakage from skin ruptures in cooked berries entails the relatively unrestricted diffusion of pigments and other solutes from epidermal cells in the exocarp and the disorganized cell layers of the exposed surface. The greatly enhanced rate of leakage with cooked berries as compared to raw samples is evidence for the destruction by heat of the primary barrier to anthocyanin diffusion from the exocarp, the semipermeable plasma membranes of epidermal cells (Adams and Blundstone, 1971). Disruption of the epicuticular wax during cooking, indicated by the appearance of a wax film on the surface of the cooling water and water in which berries were stirred, as well as by the loss of bloom, also would remove a barrier to anthocyanin leakage from underlying tissues.

Cultivar differences in the rate of anthocyanin leakage (Table 2) were large, Bluecrop and Coville leaking less than Burlington and Elliott. If anthocyanin leakage were controlled by diffusion from the pigment-containing epidermal cells through the overlaying cuticle over the entire berry surface, one would expect leakage rates to be directly proportional to the berry surface area and anthocyanin content. Although these characteristics varied among the cultivars compared, correlations between them and leakage rates were marginally significant, surface area appearing to be less important than total anthocyanin (Table 3). The apparent inverse relationship between leakage rates and the SS/A ratio, an indicator of ripeness (Woodruff et al., 1960), may be fortuitous since the low ratio for Elliott reflects the high acidity that is characteristic of this cultivar rather than a lack of ripeness (Sapers et al., 1984a). The incidence of skin rupturing does not appear to explain differences in leakage rates between cultivars (see Table 2, Bluecrop vs Coville). However, the extent of skin rupturing, i.e., the dimensions of the rupture or exposed skin undersurface, which were not measured, may be more directly related to leakage than the incidence of skin rupturing. Both the incidence and severity of skin rupturing after cooking may be related to the thickness, tensile strength, and condition (damage due to weathering or handling) of the berry skin.

The occurrence of two-phase linear absorbance vs time curves suggests a change in the mechanism of leakage during the period of measurement, perhaps the depletion of one pigment source (i.e., a skin rupture) with subsequent diffusion from a less accessible pigment source (i.e., diffusion from epidermal cells through the cuticle) determining the leakage rate. The correlation between slope 2 and the total anthocyanin content and surface area is consistent with diffusion over the entire berry surface.

Both mechanisms, anthocyanin leakage through the cuticle and from skin ruptures, probably contributed to the coloration of cooking water, as observed previously (Sapers et al., 1984b). Such leakage may be desirable with canned products, for which a highly colored syrup is sought, but would be undesirable with baked products where diffusion of anthocyanins through the batter would produce an unattractive appearance. Cultivars such as Bluecrop and Coville would be superior to Burlington and Elliott for the latter application. Information on the causes of cultivar differences in the tendency of cooked blueberries to leak may be useful in selecting new cultivars for superior processability.

Table 1—Anthocyanin leakage vs skin rupturing in steam-cooked blueberries

Cultivar	Trial	Berries with skin ruptures (%)	Leakage rate (X10^{-3})[a] Slope 1	Slope 2
Elliott	1	27	71.0	—
	2	32	73.4	46.6
	3	38	64.6	—
	4	52	125.2	61.0
	5	62	158.0	87.2
Jersey	1	7	9.2	24.2
	2	21	44.0	—
	3	49	87.0	60.8
	4	50	89.2	45.6
	5	58	70.8	22.6

[a] Absorbance units per min per 100g berries

Table 3—Correlation between leakage rate and berry total anthocyanin content and surface area

Correlation	Correlation coefficient Slope 1	Slope 2
Slope vs surface area	0.400	0.560[b]
Slope vs TAcy[a]	0.824	0.878[b]
Slope vs surface area x Tacy	0.879[b]	0.923[b]

[a] Total anthocyanin content
[b] Significant at p = 0.05

Table 2—Anthocyanin leakage from steam-cooked blueberries

Cultivar	SS/A[a]	TAcy[b]	Total surface area (cm^2)	Berries with ruptures (%) Mean	Range	Leakage rate (X10^{-3})[c] Slope 1 Trials	Mean	Range	Slope 2 Trials	Mean	Range
Bluecrop	21.0	66	166	15	12–18	3	54.3[e]	47.8– 62.6	4	25.0[f]	15.0–35.4
Burlington	13.5	152	230	44	35–53	4	95.6[d]	79.6–111.0	4	54.2[de]	38.0–64.4
Coville	22.0	143	186	58	38–77	4	66.4[e]	56.0– 77.2	5	30.5[f]	22.4–42.8
Elliott	11.9	233	207	42	27–62	5	98.4[d]	64.6–158.0	3	64.9[d]	46.6–87.2
Jersey	20.9	164	221	44	21–58	4	72.8[e]	44.0– 89.2	3	43.0[e]	22.6–60.8

[a] Soluble solids/titratable acidity
[b] Total anthocyanin = absorbance of ethanolic extract at 543 nm x dilution factor
[c] Absorbance units per min per 100g berries
[d-f] Means with different superscripts in same column are significantly different (p < 0.05)

against the applied stress (σ). In the linear viscoelastic region, the emulsion behaved essentially like a Hookean solid and stress-strain relationship was linear. The shear modulus G is given by σ/γ. Thus, the shear modulus was obtained from the slope of γ-σ plot. The stress-strain behavior of the A series emulsions and xanthan gum solutions is given in Fig. 2 and 3, respectively. The B series emulsions (except for the one with 70% oil) did not exhibit creep-recovery behavior. The emulsion with 70% oil (without xanthan gum) was viscoelastic to some extent but the precise measurement of shear modulus was not possible due to the low values of stresses involved. Table 1 lists the shear modulus values for the emulsions and solutions.

The shear modulus for the emulsions is an order of magnitude higher than that for the corresponding xanthan gum solutions. If the emulsions are regarded as the xanthan gum microgel filled with the emulsion droplets (soya oil droplets with the emulsifier molecules adsorbed at the interface), there will be a reinforcing effect due to the emulsion droplets. However, one would expect this effect to be very small, since the shear modulus of the emulsion droplets without the xanthan gum microgel is negligible (none was observed for the B series emulsions). To obtain an order of magnitude reinforcing effect as in the present case, the modulus

of the filler droplets would have to be quite high. Thus the composite theories such as Kerner model (Kerner, 1956) or van der Poel model (Poel, 1958) cannot adequately explain the behavior of the emulsions.

The xanthan gum solutions and the emulsion (whtout xantham gum) separately have low shear moduli. However, when they were mixed together as in the A series emulsions, it resulted in a large increase in the shear modulus. This is probably due to the strong interactions between the emulsion droplets and xanthan microgel. One possibility is that the xanthan gum molecules get adsorbed at the oil-water interface. There is evidence in the literature to suggest that xanthan gum may be surface active (Prud'homme and Long, 1983). Xanthan gum may act partly as an emulsifier (by being adsorbed at the interface) and partly as a thickener (by forming a microgel). There may also be interactions between xanthan gum molecules and egg yolk lipoproteins.

Fig. 4b shows the plot shear modulus vs volume fraction of oil. The dependence is linear over the entire range studied with a slope of 1.6. According to the theory proposed by Buscall et al. (1982), shear modulus G is related to the total interparticle potential V by

$$G = \frac{\alpha}{R}\left(\frac{d^2 V}{dr^2}\right)_{r=R} \qquad (1)$$

where, R is the equilibrim center to center distance and α is a constant. Assuming that the dominant repulsive interaction between the droplets is represented by an inverse power potential, $[V(r) \alpha\ r^{-n}]$, the relationship between G and ϕ, the disperse phase volume fraction can be obtained as follows (Gladwell et al., 1985):
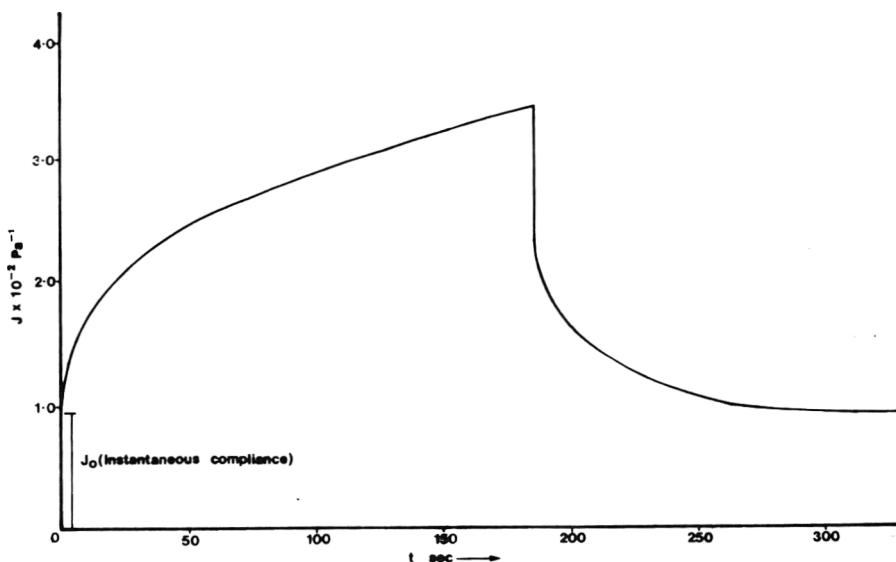
$$\log G = \left(\frac{n}{3} + 1\right) \log \phi + \text{constant} \qquad (2)$$

According to a recent article by Sornette and Ostrowsky (1984), the steric interaction between fluctuating spherical drops gives a relationship of the form

$$G \alpha\ \phi^{4/3}\ [1 - (\phi/\phi_m)^{1/3}]^{-3} \qquad (3)$$

where ϕ_m is the close packed volume fraction. For $\phi \ll \phi_m$ this gives an exponent 4/3. However, for larger values of ϕ there is no simple exponent although if a correlation is forced, the effective exponent will be greater than 4/3. Our results, with the power of 1.6 are consistent with this approach. This suggests that the interaction between the

Table 1—Composition and shear moduli of oil-water emulsions and xanthan gum solutions. A1 to A6 are emulsions, while X1 to X6 are xanthan gum solutions[a]

Sample	Water wt %	Soya oil wt %	Egg yolk wt %	Xanthan wt %	Droplet size μm	Shear modulus
						Pa
A1	76	20	3	1	2.4	107
A2	66	30	3	1	3.1	176
A3	56	40	3	1	3.0	295
A4	46	50	3	1	2.7	395
A5	36	60	3	1	2.8	562
A6	26	70	3	1	2.9	957
X1	98.7	–	–	1.3	–	6.00
X2	98.5	–	–	1.5	–	8.62
X3	98.2	–	–	1.8	–	11.8
X4	97.8	–	–	2.2	–	21.6
X5	97.2	–	–	2.8	–	35.0
X6	96.2	–	–	3.8	–	44.5

[a] The B series emulsions had compositions identical to the A series emulsions except that no xanthan gum was used.

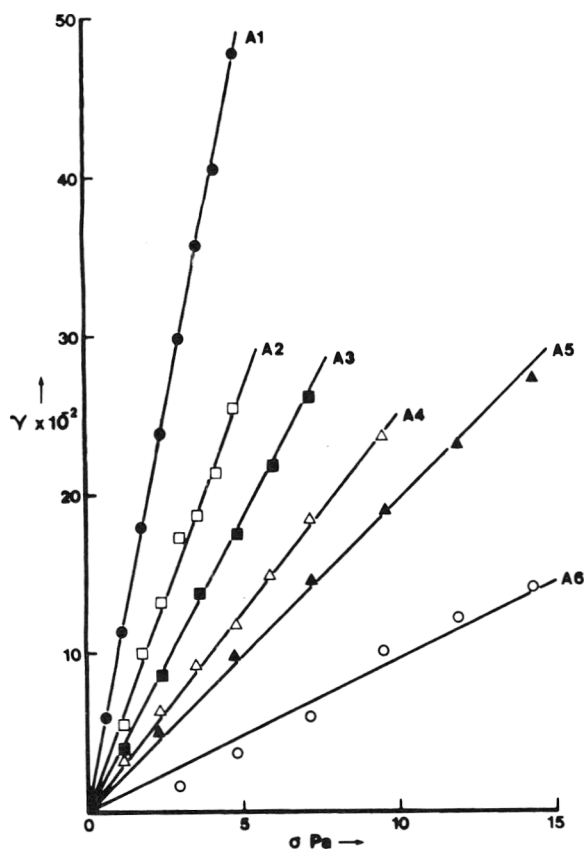Fig. 1—A typical compliance curve for the Al emulsion. σ = 2.38 Pa.

Fig. 2—Strain-stress behaviour of the A series emulsions. γ - shear strain, σ - shear stress, Pa.
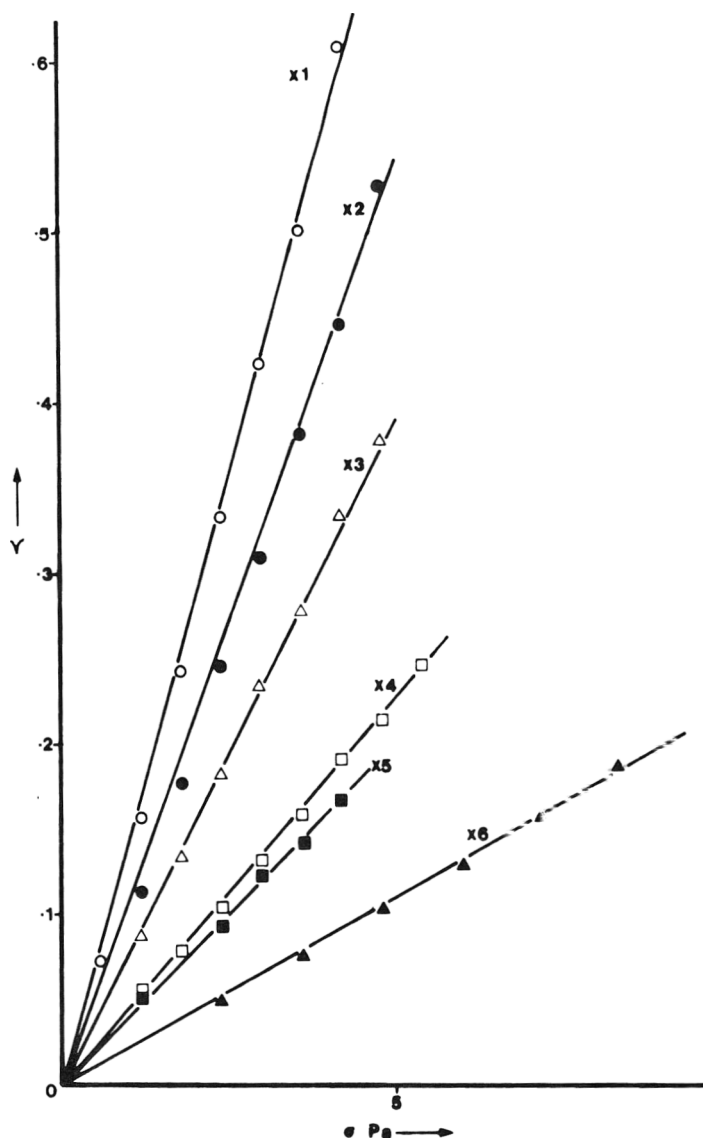

Fig. 3—Strain-stress behavior of xanthan gum solutions.

droplets is similar to that between the oscillating spheres rather than that between the rigid spheres. This will be the case if the droplets deform easily under the applied shear stress. Network or chain models for the flocculated emulsions predict a linear relationship between G and ϕ (van den Tempel, 1961; Nederveen, 1963; Sherman, 1970). However, this is contrary to some experimental observations. A much stronger dependence of G upon ϕ was observed by Sherman (1967) and by Hoffman and Myres (1965). G was found to be dependent upon ϕ^2 by Zosel (1982) for polystyrene latex suspensions and for TiO_2 suspensions in water. He postulated the presence of ϕ_c, a critical disperse phase concentration below which G = 0. However, in our case, there was no ϕ_c, since even at $\phi = 0$, the system (xanthan solution when $\phi = 0$) had a measurable shear modulus.

Plot of log G vs log C for xanthan solutions is also a straight line with the slope of 2 (Fig. 4a). This is roughly similar to the log G - log ϕ relationship for the emulsions. Xanthan gum solutions were prepared at room temperature. Xanthan gum solutions prepared in this way are thought to be dispersions, rather than the true solutions (Dintzis et al., 1970). Increasing the xanthan gum concentration also increases the concentration of the xanthan

gum particles in the dispersion. According to Morris et al. (1983) this is the origin of the shear modulus for xanthan gum solutions. When the solutions were heated to obtain true solutions and then cooled again, the shear modulus disappeared and the solutions exhibited Newtonian behavior. If most of the increase in G can be attributed to the presence of particulate matter in the xanthan gum solutions', one would expect the G-C dependence to be similar to the G-ϕ dependence for the emulsions, thus explaining our results.

CONCLUSIONS

IN SOYA OIL-WATER EMULSIONS most of the viscoelastic behavior can be attributed to the interactions between
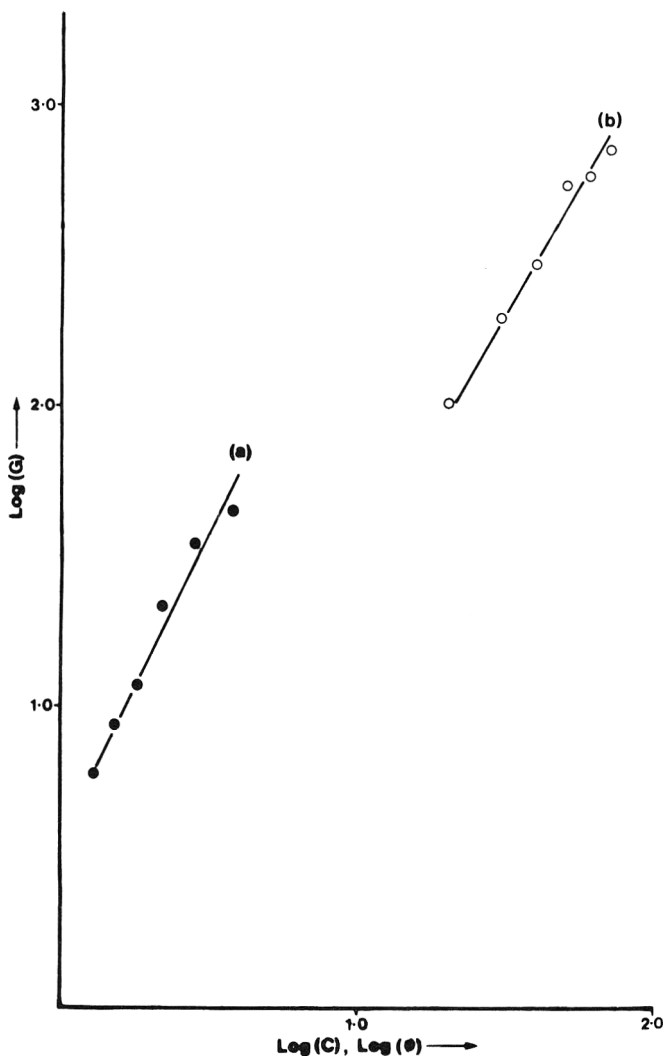
Fig. 4—(a) Plot of shear modulus vs concentration for the xanthan solutions; (b) Plot of shear modulus vs volume fraction of oil for the emulsions. G - shear modulus, Pa; c - concentration, weight %; φ - volume percent of oil.

xanthan gum microgel and emulsion droplets. The results obtained do not agree with the predictions of the chain or network models, but are in agreement with the theory of membranes, indicating that the droplets behave as soft deformable spheres, rather than as rigid spheres. While xanthan gum solutions are much less viscoelastic than the emulsions, their behaviour follows a similar pattern, indicating that the xanthan gum solutions prepared at room temperature are probably dispersions and not true solutions.

REFERENCES

Buscall, R., Goodwin, J.W., Hawkins, M.W., and Ottewill, R.H. 1982 Viscoelastic properties of concentrated lattices. Part 2 — Theoretical analysis. J. Chem. Soc. Faraday Trans I. 78: 2889.
Dickinson, E. and Stainsby, G., 1982. "Colloids in Food." Applied Science Publishers, London.
Dintzis, F.R., Babcock, G.E., and Bosin, R. 1970. Studies on dilute solutions and dispersions of the polysaccharide from Xanthomonas campestris NRRL B-1459. Carbohydrate Res. 13: 257.
Gladwell, N., Grimson, M.J., Rahalkar, R.R., and Richmond, P. 1985. Linear viscoelastic behaviour of sterically stabilized oil-water emulsions. J. Chem. Soc. Faraday Trans. In press.
Hennock, M., Rahalkar, R.R., and Richmond, P. 1984. Effect of xanthan gum upon the rheology and stability of oil-water emulsions. J. Food Science. 49(5): 1271.
Hoffman, R.D. and Myres, R.R. 1965. Dynamic mechanical properties of some non-aqueous dispersions. Proc. 4th Int. Cong. Rheol. 2: 693.
Kerner, E.H. 1956. The elastic and thermo-elastic properties of composite media. Proc. Phys. Soc. 69: 808.
Morris, V.J., Franklin, D., and I'Anson, K. 1983. Rheology and microstructure of dispersions and solutions of the microbial polysaccharide from Xanthomonas compestris (xanthan gum). Carbohydrate Res. 121: 13.
Nederveen, C.J. 1963. Dynamic mechanical behaviour of suspensions of fat particles in oil. J. Colloid Sci, 18: 276.
Poel, P. van der 1958. On the rheology of concentrated dispersions. Rheol. Acta. Band 1, Nr. 2-3, 198.
Prud'homme, R.K., and Long, R.E. 1983. Surface tensions of concentrated xanthan and polyacrylamide solutions with added surfactants. J. Colloid Interface Sci. 93: 274.
Sherman, P., 1970. The influence of particle size on the viscoelastic properties of flocculated emulsions. Proc. 5th Int. Cong. Rheol. 2: 327.
Sherman, P. 1967. Rheological changes in emulsions on aging. III. At very low rates of shear. J. Colloid Interface Sci. 24: 107.
Sornette, D. and Ostroweky, n. 1984. Repulsive steric interaction between membranes of finite size. J. Physique 45: 265.
Tempel, M. van den, 1961. Mechanical properties of plastic-disperse systems at very small deformations. J. Colloid Sci. 16: 284.
Whorlow, R.W. 1980. "Rheological Techniques." J. Wiley. London.
Zosel, A. 1982. Rheological properties of disperse systems at low shear stresses Rheol. Acta 21: 72.
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CONCLUSIONS

Significant anthocyanin leakage from raw blueberries does not occur, except in stored berries that have undergone softening. The removal of the epicuticular wax has little effect on the extent of anthocyanin leakage from raw berries.

Cooking destroys the primary barrier to anthocyanin leakage, resulting in high leakage rates. Cultivar differences in anthocyanin leakage rates after cooking are due primarily to differences in the tendency of berry skins to rupture, and secondarily to berry anthocyanin content and surface area.

REFERENCES

Adams, J.B. and Blundstone, H.A.W. 1971. Canned fruits other than citrus. Chapter 15 in "The Biochemistry of Fruits and their Products, Vol. 2, Part 2. The Biochemistry of Fruit Processing," (Ed.) A.C. Hulmes. Academic Press. New York.
Albrigo, L.G., Lyrene, P.M., and Freeman, B. 1980. Waxes and other surface characteristics of fruit and leaves of native Vaccinium elliotti Chapm. J. Amer. Soc. Hort. Sci. 105: 230.
Ballinger, W.E., Kushman, L.J., and Hamann, D.D. 1973. Factors affecting the firmness of highbush blueberries. J. Amer. Soc. Hort. Sci. 98: 583.
Dekazos, E.D. and Smit, C.J.B. 1976. Effect of variety, packaging and storage conditions on the shelf life and quality of rabbiteye blueberries. Georgia Agric. Res. 18(2): 19.

Hodgman, C.D. 1954. "Handbook of Chemistry and Physics," 36th ed. Chemical Rubber Publishing Co., Cleveland, OH.
Joslyn, M.A. 1966. The freezing of fruits and vegetables. Ch. 12. In "Cryobiology," (Ed.) H.T. Meryman. Academic Press, New York.
Norris, R.F. 1974. Penetration of 2,4-D in relation to cuticle thickness. Amer. J. Botany 61: 74.
Sapers, G.M., Burgher, A.M., Phillips, J.G., and Jones, S.B. 1984a. Color and composition of highbush blueberry cultivars. J. Amer. Soc. Hort. Sci. 109: 105.
Sapers, G.M., Burgher, A.M., Phillips, J.G., and Jones, S.B. 1984b. Effects of freezing, thawing and cooking on the appearance of highbush blueberries. J. Amer. Soc. Hort. Sci. 109: 112.
Sapers, G.M., Phillips, J.G., Rudolf, H.M., and DiVito, A.M. 1983. Cranberry quality: Selection procedures for breeding programs. J. Amer. Soc. Hort. Sci. 108: 241.
Scheffe, H. 1953. A method for judging all contrasts in the analysis of variance. Biometrika 40: 87.
Schönherr, J. 1976. Water permeability of isolated cuticular membranes: The effect of cuticular waxes on diffusion of water. planta (Berl.) 131: 159.
Woodruff, R.E., Dewey, D.H., and Sell, H.M. 1960. Chemical changes of Jersey and Rubel blueberry fruit associated with ripening and deterioration. Proc. Amer. Soc. Hort. Sci. 75: 387.
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Effect of Hydration of Cowpea Meal on Physical and Sensory Attributes of a Traditional West African Food

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ABSTRACT

Effects of water level (56, 58 and 60%) and hydration time (0, 30, and 60 min) on apparent viscosity of cowpea paste and physical-sensory attributes of akara, a finished food made from paste, were determined. Water level had a greater effect than hydration time on paste viscosity and on crude fat content, shear force, lightness (L), and saturation index (ΔE) of akara. Apparent viscosity of cowpea paste was the only parameter significantly affected by the interaction of water level and hydration time. Overall, the 60% water level produced paste with flow properties and akara with physical characteristics most like the traditional product. Sensory attributes of meal-based products were acceptable.

INTRODUCTION

COWPEAS (*Vigna unguiculata*) are widely consumed in West Africa as a boiled vegetable using fresh or rehydrated dry seeds, as an ingredient in soups, stews, and casseroles, and as paste in steamed and fried dishes (Dovlo et al., 1976). Two time-consuming processes involved in preparing cowpeas for certain applications are removal of the seed coat and size reduction of seed. In an effort to simplify processing of cowpeas and other grain legumes in developing countries, village-scale technologies of decortication (Reichert and Youngs, 1976; Reichert et al., 1979) and flour production (Anon., 1976; Onayemi and Potter, 1976; Eastman, 1980) have been developed.

A collaborative research project being conducted by the University of Georgia and the University of Nigeria under the auspices of the Bean/Cowpea Collaborative Research Support Program (U.S. Agency for International Development) is seeking to improve the processing, preservation, and utilization of cowpeas. In addition to developing technologies to improve the ease and efficiency of removing the cowpea seed coat, a major focus of the project has been optimizing conditions for processing and using cowpea meal. Although cowpea flour is available in West African markets, it has not gained widespread acceptance, largely because of its poor functionality, i.e., water absorption and performance in preparation of traditional foods (Dovlo et al., 1976). A recent study in our laboratory determined that the poor performance of a commercial cowpea flour obtained from Nigeria and used as an ingredient in akara, a traditional West African dish made by deep-fat frying cowpea paste, was related to its small average particle size (McWatters, 1983). Particle size analysis of the flour showed the greatest concentration, 48%, of particles to be in the 400-mesh range whereas the greatest concentration of traditionally made paste particles, 42%, was in the 100-mesh range. This indicated that milling conditions presently employed in West Africa for producing fine flours from maize and sorghum may not be appropriate for cowpeas. Williams (1980) concluded that cowpea meal (middlings) was more suitable for akara preparation than fine flour or coarse grits.

In addition to particle size distribution, other factors to be considered in determining and optimizing the performance of cowpea meal or flour are water:solids ratio and hydration time. One Nigerian manufacturer's instructions for using cowpea flour recommended a flour to water ratio of 4:5 and no hydration time whereas another recommended a 4:3 flour to water ratio and a 3-hr hydration time. Since recommended procedures for utilizing cowpea meal/flour in traditional West African foods are quite variable, this study was conducted to determine the influence of hydrating cowpea meal under different conditions on its functional performance in a specific food system, i.e., akara.

MATERIALS & METHODS

Cowpea meal and paste preparation

The California blackeye pea was selected as the seed source because of its acceptability in developing countries including Nigeria, Senegal, Botswana, and the West Indies; Nigeria has imported California blackeye peas in recent years because of insufficient domestic supplies (Hall, 1984). Most of the dry seed industry in the U.S. also uses blackeye-type cultivars, usually California Blackeye 5 (Fery, 1981).

Peas (4.5 kg) were decorticated for 6 min in a mini-PRL rollover dehuller (Nutana Machine Co., Saskatoon, Canada) equipped with carborundum stones rotating at 1,000 rpm. After passing through a seed cleaner, decorticated cotyledons were made into meal in a Retsch microjet ultracentrifugal mill, Model ZM1, equipped with a 1.0 mm screen and operated at 10,000 rpm. Previous work had shown that the 1.0 mm screen produced a particle size distribution in meal which resulted in acceptable end product quality (McWatters, 1983). The moisture content of the meal, determined by vacuum drying 5-g samples for 24 hr at 70°C, was 11%. Paste was prepared by adding sufficient water to the meal to adjust the moisture content to 56, 58, or 60%. Preliminary studies had shown that water levels below 56% or above 60% produced pastes that had poor flow characteristics, i.e., were too thick or thin, respectively. For each batch, 200g cowpea meal was mixed with water and stirred gently with a spoon for 2 min. The resulting paste was covered and held undisturbed for 0, 30, or 60 min. Each water level-hydration time treatment was replicated twice.

Akara preparation

Akara was prepared from pastes made from the water level/hydration time treatments described above. Akara was also prepared by the traditional process (Dovlo et al., 1976) for comparison. Seasoning levels and frying conditions associated with akara preparation have been described previously (McWatters and Brantley, 1982; McWatters, 1983).

Physical measurements

Viscosity of pastes whipped in a Hobart mixer (N-50) at speed 3 for 1.5 min was determined. Apparent viscosity of pastes at 23°C was measured with a Brookfield Viscometer Model RVT and Model C Helipath Stand equipped with a TB spindle and operated at 5 rpm (Chhinnan et al., 1983). The dial readings of the viscometer were multiplied by the manufacturer-supplied conversion factor of 8.0 to obtain values in poise.

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Akara was analyzed in quadruplicate for moisture content, crude fat content, and force required to shear as described by McWatters (1983). Hunter color values (L, a, b) of four akara balls from each treatment were determined with a Gardner XL-845 colorimeter set against a yellow reference standard (L = 78.53, a = -2.03, b = 23.55). Psychometric color terms involving chroma (C), chromaticity difference (ΔC), hue difference (ΔH) and saturation index (ΔE) were calculated from L, a, b values as given below (Anon., 1979):

$$C = (a^2 + b^2)^{1/2}$$
$$\Delta C = (a_s^2 + b_s^2) - (a_r^2 + b_r^2)^{1/2}$$
$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$
$$\Delta H = [(\Delta E)^2 - (\Delta L)^2 - (\Delta C)^2]^{1/2}$$

where, Δ refers to the difference between sample and reference standard; subscripts r and s are associated with the reference standard and the sample, respectively. The L, a, b color space and the above psychometric color terms are represented graphically in Fig. 1.

Sensory evaluation

A set of four samples consisting of a reference (R) made by the traditional process and the products of three hydration times at one water level was evaluated at each session. Samples for each set were prepared in the morning, cooled to room temperature, and covered with aluminum foil until evaluated at mid-afternoon. Samples were arranged in random order on heat-resistant white plates, reheated at 150°C for 5 min in a conventional oven, and evaluated while warm by a ten-member panel. All panelists had served on previous akara panels, were familiar with the traditional product, and were trained in the use of sensory evaluation procedures. Samples were rated for color, texture, and flavor on a scale of 9 to 1 (9 = excellent, 5 = borderline, 1 = very poor) as described by McWatters (1983).

Statistical analysis

The objective and sensory data were analyzed separately using analysis of variance procedure, PROC ANOVA, of Statistical Analysis System - SAS 79 (Helwig and Council,

1979). The objective measurement variables associated with paste and akara prepared from cowpea meal were first analyzed as a function of hydration time and water level. Following this analysis, appropriate hypotheses were tested to compare the difference between akara prepared from cowpea meal and by the traditional process.

For sensory data analysis, the reference scores corresponding to each set were analyzed to test their variation among sets. No statistical difference at the 5% significance level was found. As a result of this, all of the sensory data was pooled and analyzed in the manner described for the objective data.

RESULTS & DISCUSSION

MOST OF THE SAMPLES tested were prepared from cowpea meal while others were prepared by the traditional process. In the following discussion, no reference to the sample preparation process will imply that the samples were prepared from cowpea meal.

Analysis of variance of the effect of water level and hydration time on paste viscosity and several physical characteristics of akara is shown in Table 1. Water level had a major effect on apparent viscosity of cowpea paste and on crude fat content, shear force, lightness (L) and saturation index (ΔE) of akara. Hydration time had a significant effect on hue difference (ΔH) of akara. Water level-hydration time interaction had a significant effect on the apparent viscosity of cowpea paste. The moisture content of akara was not affected by either water level or hydration time.

Data for traditionally processed cowpea paste and akara and those obtained for cowpea meal were pooled. The pooled apparent viscosity data were analyzed considering each water level and hydration time as 9 individual treatments and traditional paste as one additional treatment (Table 2). This approach was taken because of significant interaction between water level and hydration time. Analysis of variance of the pooled data for moisture content, crude fat, shear force, L, and ΔE values considering each water level of cowpea meal as an individual treatment and the traditionally prepared akara as the fourth treatment is shown in Table 3. Hue difference, ΔH, was found to be affected by hydration time only (Table 1), and pertinent analysis of the pooled data for this color characteristic is presented in Table 4.

The first step in analysis of the sensory data involved testing the variability in the reference scores given by the panelists on different days of scoring. It was found that no statistical difference existed among different sets of reference scores. It may be noted that the reference score was the score assigned by a panelist to the akara sample prepared by the traditional process. Statistical analysis of the sensory attributes of akara for the data corresponding to all combinations of water level and hydration time was performed. No significant effect on the sensory attributes of color, texture, and flavor due to these treatments was observed. Results from the analysis comparing sensory attributes to akara made from cowpea meal and by the traditional process are given in Table 5.

Pastes prepared at the 56% water level had the highest apparent viscosity values and were the most viscous, 58% water level pastes were intermediate, and 60% water level pastes were the least viscous (Table 2). At the 56% water level, viscosities of pastes hydrated for 30 and 60 min were similar and significantly less viscous than paste at the 0 min hydration time. At the 58% water level, viscosities of pastes hydrated for 0 and 30 min were similar and significantly more viscous than paste hydrated for 60 min; the same trend was observed at the 60% water level. All treatments produced pastes which were more viscous than paste made by the traditional process. Overall, the 60% water level pro-
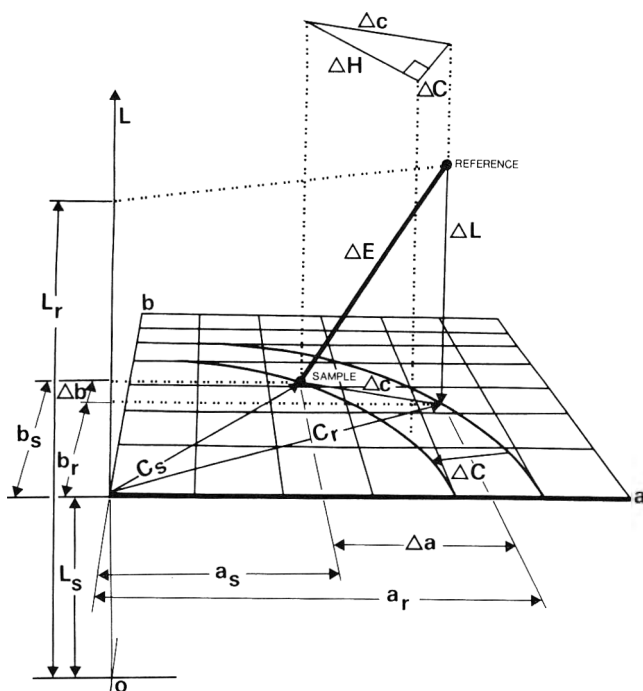

Fig. 1—Graphic representation of psychometric color terms.

Table 1—Level of significance for the main and interactive effects of water level and hydration time on physical variables associated with cowpea paste and akara prepared from cowpea meal

	Cowpea paste	Akara					
	Apparent viscosity (Poise)	Moisture (%)	Crude fat (%)	Shear force (N/g)	Instrumental color values		
					L	Δ E	Δ H
Water Level, W (%)	•••	NS	•••	•••	•	•••	NS
Hydration time, T (min)	NS	NS	NS	NS	NS	NS	•••
W × T	•••	NS	NS	NS	NS	NS	NS

NS - not significant; •P≤0.05; ••P≤0.01; •••P≤0.001

Table 2—Effect of water level and hydration time on apparent viscosity of whipped cowpea paste prepared from cowpea meal and traditional paste

Water level (%)	Hydration time (min)	Apparent viscosity (poise)
56	0	578.4[a]
	30	559.2[b]
	60	563.2[b]
58	0	440.8[c]
	30	436.0[c]
	60	423.2[d]
60	0	332.8[e]
	30	321.6[e]
	60	316.0[f]
Traditional paste		301.6[g]

[a-g] Values not having a common superscript are significantly different at P ≤ 0.05.

Table 3—Effect of water level on moisture content, crude fat content, shear force, L, and ΔE values of akara prepared from cowpea meal and traditional paste

Process	Water level (%)	Moisture (%)	Crude fat[c] (%)	Shear force (N/g)	L	Δ E
Cowpea meal	56	46.8	24.2[d]	14.6[a]	38.3[a]	41.7[b]
	58	47.1	26.4c	13.5[b]	38.0[a]	42.1[b]
	60	47.5	29.0[b]	11.7[c]	35.2[b]	44.8[a]
Traditional		45.4	38.6[a]	12.5[bc]	35.8[b]	44.3[a]

[a-d] Values in a column not having a common superscript are significantly different at P ≤ 0.05.

[e] Dry weight basis.

Table 4—Effect of hydration time on hue difference, ΔH, of akara prepared from cowpea meal and traditional paste

Process	Hydration time (min)	Δ H
Cowpea meal	0	8.96[c]
	30	9.64[b]
	60	10.41[a]
Traditional	-	10.62[a]

[a-c] Values not having a common superscript are significantly different at P ≤ 0.05.

Table 5—Comparison of sensory ratings[1] of akara prepared from cowpea meal and traditional paste

Process	Color	Texture	Flavor
Cowpea meal	7.61[a]	6.92[b]	7.30[b]
Traditional	7.85[a]	7.95[a]	7.95[a]

[a-b] Scale of 9 to 1 where 9 = excellent, 5 = borderline, 1 = very poor; values in a column not having a common superscript are significantly different at P ≤ 0.05.

duced paste viscosities most like that of traditional paste (61.3% moisture).

In a previous study (Chhinnan et al., 1983) involving rheological characteristics of cowpea paste, the authors had found that the apparent viscosity of cowpea paste was significantly affected by water level only; however, the magnitude of viscosity values reported are comparable to those obtained in this study.

Water level had a major effect on crude fat content and shear force but not on moisture content of akara prepared from cowpea meal (Table 3). Percentage crude fat increased as the level of water used to hydrate cowpea meal increased

from 56 to 60%. All water level treatments produced akara which was significantly lower in crude fat content than akara made by the traditional process. Shear force values indicate that the amount of force required to shear akara decreased significantly as water level for meal hydration increased from 56 to 60%. Shear values for the 58 and 60% water levels were similar to that of traditionally-made akara and significantly lower than shear values obtained at the 56% water level.

Color values for lightness (L) and saturation index (ΔE) of akara were also influenced by the water level used to hydrate cowpea meal (Table 3). Akara balls prepared from meal at the 56 and 58% water levels had similar L values and were significantly lighter than those at the 60% water level or those made by the traditional process. Saturation index or total color difference values indicate that the 60% water level produced akara more like the traditional product than the 56% or 58% water levels. Hue difference (ΔH)values of akara increased as the length of time for hydrating cowpea meal increased from 0 to 60 minutes (Table 4). Akara made from cowpea meal hydrated for 60 minutes and akara made by the traditional process had similar ΔH values.

Comparison of sensory scores for akara prepared from hydrated cowpea meal and from traditionally processed paste indicates that there was no significant difference in color due to processing treatment (Table 5). Texture and flavor scores of akara prepared from hydrated meal were significantly lower than those of the traditional product. All of the meal-based products were acceptable, with mean scores of 7 (good) for color and flavor and 6 (fair) for texture. A frequent comment made by panelists concerning texture was that the test products were drier and less oily than traditional akara. Since the moisture content of traditional and test samples were similar (Table 3), the perceived drier texture of the meal products was probably due to their lower crude fat content.

Results of this study show that the level of water used to hydrate cowpea meal had a greater effect on physical characteristics of paste and akara than the length of time the meal was hydrated or the interaction of water level and hydration time. Overall, the 60% water level produced paste with flow properties and akara with physical characteristics more like the traditional product than the other water levels. Sensory attributes of all meal-based products were acceptable, receiving ratings of good (color, flavor) or fair (texture). Optimizing conditions for processing and using cowpea meal should promote the resourceful utilization of this important legume.

REFERENCES

Anonymous. 1976. Maiduguri mill project. International Development Research Center, Bull. IDRC-TS2, p. 1.
Anonymous. 1979. CIE dimensions for hue and hue-difference and shade sorting with Tektronix 31 and D25-9 Shademaster. Hunterlab Reflections & Transmissions 30: 4.
Chhinnan, M.S., McWatters, K.H., Rao, V.N.M., and Phillips, R.D. 1983. Rheological properties of cowpea paste. Proc. Sixth Int. Congr. of Food Science and Technology, Dublin, Ireland. 2: 132.
Dovlo, F.E., Williams, C.E., and Zoaka, L. 1976. Cowpeas: Home preparation and use in West Africa. International Development Research Center, Bull. IDRC-055e, p. 1.

Kinetics of Folacin Destruction in Swiss Chard During Storage

D. B. GAMI and T.-S. CHEN

ABSTRACT

The folacin activities in fresh Swiss chard leaves stored in open air at 4, 21, 35 and 40°C were determined by a microbiological assay using *Lactobacillus casei*. At 21°C the leaves were also stored in plastic bags and under moist conditions. Folacin was most stable when the vegetable was stored in plastic bags, followed by the moist condition, and least stable in open air at 21°C. The degradation of folacin in Swiss chard under all conditions followed first-order kinetics. The temperature dependent folate degradation conformed to the Arrhenius equation and the activation energy was 24 kcal/mole.

INTRODUCTION

FOLACIN DEFICIENCY is probably the most common vitamin deficiency in the U.S. (Chanarin, 1981). The USDA Nationwide Food Consumption Survey, 1977-78, indicated that the RDA for folacin is difficult to meet and the estimated average folacin intake for men and women is 88% and 71% of the RDA, respectively (Peterkin et al., 1981). Quantitative assessment of folacin status through dietary intake information, however, has been exceedingly difficult. The problem is mainly a lack of reliable food folate data. Many foods that may contribute to dietary folacin intake have not been assayed by the recognized *Lactobacillus casei* microbiological method and the effects of storage, processing and preparation on folacin in these foods have not been studied.

Green leafy vegetables are generally considered good sources of folacin. Swiss chard is a garden vegetable plant with large fleshy curly or fanlike leaves and broad midribs which could be white or red. The plant withstands dry weather well and grows constantly throughout the summer. A search of literature reveals two reports on folacin in Swiss chard. Olsen et al. (1947) reported 111 µg of folacin per 100g chard but the value may be underestimated since ascorbic acid was not used in the assay to protect folacin. Mullin et al. (1982) determined folacin content of three cultivars of Swiss chard and the storage effect at 4°C.

Interest in quantitative approaches to analysis of food quality deterioration during processing and storage has resulted in numerous kinetic studies on nutrient destruction. Since 1979 when Chen and Cooper (1979) reported the kinetics of thermal destruction of two naturally occurring folates, several kinetic studies on folacin degradation reactions involving heating synthetic folacin derivatives in buffers or in model food systems have been published (Day and Gregory, 1983; Mnkeni and Beveridge, 1982, 1983). However, the destruction kinetics of this vitamin as a natural food component have not been previously investigated. The purposes of this study were to determine the effect of storage conditions on the stability of folacin and the temperature dependence of the rate of folacin degradation in Swiss chard.

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MATERIALS & METHODS

Sample

Fresh Swiss chard (*Beta vulgaris*; var. cicla), with broad white midribs and fanlike leaves, grown in Oxnard, CA, was obtained from a wholesale distributor within 48 hr of harvest. The lower stems of the leaves were removed and the leaves washed and patted dry on paper towels. Preliminary experiments showed that folacin content was directly related to the leaf size. To control the variability due to leaf size, the leaves were sorted, based on weight, into six lots: 1 – 10g, 11 – 20g, 21 – 30g, 31 – 40g, 41 – 50g, and 51 – 60g. One leaf from each lot was taken to assemble a sample bunch of six leaves and a total of 72 sample bunches was assembled. The sample bunches were then divided into six groups for stroage under various conditions. Three groups were stored at 21°C, one each in open air, under moist condition and in plastic bags. The remaining three groups were stored in open air at 4, 35, and 40°C. For moist storage, a tray containing water was placed in a cardboard box (18 × 12 × 10 in with holes on the sides) and the sample bunches were placed in rows on wire racks above the water tray. The box was covered with wet cheese cloth. For storage in plastic bags, the produce bags of the clear polyethylene type available in supermarkets were used. Sample bunches were put one in each bag and the bags were sealed by twist-tieing at the neck. For open air storage, the sample bunches were placed in open cardboard boxes.

Sample extraction

A sample bunch of Swiss chard was withdrawn from each storage chamber at various time intervals for extraction and analysis of folacin activity. The leaves in each bunch were chopped and well mixed. Triplicated samples of 20g each of chopped leaves were immediately extracted in 200 mL of boiling sodium phosphate buffer (0.1M, pH 6.1) containing 0.5% ascorbic acid, by homogenizing in a Waring blender at high speed for 1.5 min. The homogenate was autoclaved at 121°C for 15 min, cooled in an ice-water bath and centrifuged at 3000 × g for 15 min at 0°C. The supernatant was assayed for folacin activity.

Folacin assay

Folacin activity of Swiss chard extract was determined by a microbiological method using *Lactobacillus casei* (ATCC 7469) as the assaying organism. To break down the polyglutamyl folacin derivatives into forms that can support the growth of *L. casei*, the sample extracts were treated with chicken pancreas conjugase. The reaction mixture, consisting of 0.5 mL of sample extract, 0.5 mL of conjugase solution and 4.0 mL buffer, was incubated at 37°C for 2 hr as described by Kirsch and Chen (1984). The conjugase-treated sample extract was then diluted 10 times with buffer and assayed in triplicate of 0.2 mL each in accordance with the method of Chen et al. (1983). The mean folacin activity and standard deviation of the mean for each sample were calculated from nine measurements. The standard deviations ranged from 0.5 – 12% of the mean values indicating low variability of the microbiological assay used. The folacin content of the vegetable, measured as folacin activity by the microbiological assay, is expressed in µg folic acid equivalent per 100 g fresh weight.

Treatment of data

The percent folacin retention in Swiss chard during storage was calculated by comparing the folacin content of stored samples to that of the fresh sample. The order of the degradation reaction of folacin during storage was ascertained graphically by plotting either the folacin content or percent folacin retention against time of storage using a computer program CURFIT based on linear

regression analysis. The straight line which resulted from plotting logarithm of folacin content or percent folacin retention versus time indicated a first order reaction. The first-order rate constant (k) was calculated from the slope of the line obtained in this semi-log plot. The half-life of folacin in Swiss chard under each storage condition was determined by the equation, $t_{1/2} = 0.693/k$.

The temperature dependence for folacin degradation in Swiss chard was further analyzed according to the Arrhenius equation:

$$k = A \exp(-Ea/RT)$$

where k = first-order rate constant; A = Arrhenius pre-exponential; Ea = Arrhenius activation energy (cal/mole); R = gas constant; and T = absolute temperature ($^\circ$K).

RESULTS & DISCUSSION

FRESH SWISS CHARD LEAVES contained 297 μg folic acid equivalents per 100g fresh weight (Fig. 1). This is significantly higher than the values of 123 – 199 μg/100g for three cultivars of field grown Swiss chard reported by Mullin et al. (1982). The difference between the values obtained in the two studies may be partially due to the difference in the experimental technique. Kirsch and Chen (1984) have shown that variations in the conjugase treatment procedures alone could result in as much as 50% variance in the folacin values assayed microbiologically. Other important factors that could affect folacin content of vegetables include variety, growing conditions, maturity and post-harvest holding conditions.

When stored at room temperature (21°C), Swiss chard retained good visual quality up to 2, 4, and 6 days under open-air, moist, and bagged conditions, respectively. The samples stored in open air were slightly discolored and wilted on the second day. The leaves became tough and the stem had brown spots on the third day. On the fourth day, the leaves turned yellow and the midrib turned brown but no disagreeable odors were noticed. The moist and bag-stored samples were crisp for the first three days of storage. The yellowing of the leaves became increasingly noticeable after four days when brown spots were also developed, more so under the moist condition. By the sixth day, the bagged sample bunch had brown spots on the middle two or three leaves of the bunch and the other leaves were yellowish green in color. Both moist and bag stored samples had developed unacceptable odor on the seventh day of storage.

The folacin content of Swiss chard stored at 21°C decreased with time under all three conditions. The bagged sample showed the slowest rate of loss followed by the
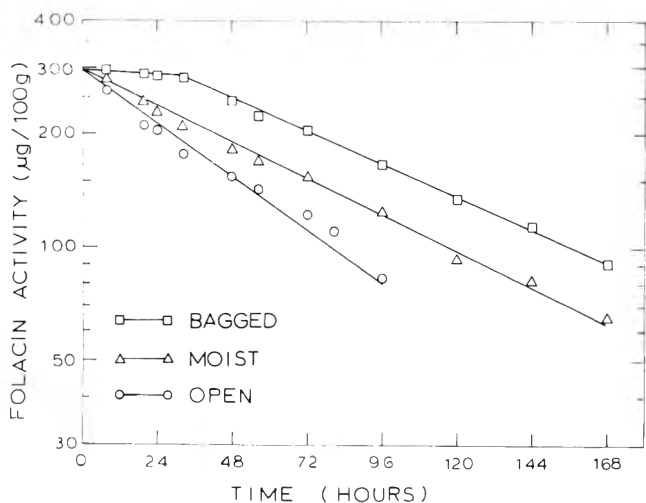
moist and open-air samples (Fig. 1). The open-air sample lost 30% folacin in 24 hr, whereas 25% and 4% losses of folacin occurred in the moist and bagged Swiss chard, respectively. As shown in Fig. 1, the linearity of the folacin degradation curves obtained on a semi-log plot indicates that the loss of folacin followed first order reaction kinetics when Swiss chard was stored under open-air and moist conditions. When stored in the plastic bag, however, two stages of the folacin degradation reaction in Swiss chard are apparent. The first part of the reaction occurs along the horizontal portion of the curve (Fig. 1) where no appreciable folacin loss is occurring. The second part of the reaction occurs along the sloped portion of the curve, after about 32 hr of storage at 21°C, and the loss of folacin could be again described by first order reaction kinetics.

The high correlation coefficients ($>$0.98) obtained from linear regression analysis indicate that the data satisfactorily conform to the first order function for the degradation of folacin in Swiss chard under all storage conditions (Table 1). The calculated first order reaction constants and half-lives are also presented in Table 1. Although the rate constant for the bagged sample was very close to that of the moist sample, plastic bags appeared to cause a delay in the initiation of folacin degradation and offered better protection for the storage of Swiss chard. Previous workers (Kenny, 1971; Umiecka, 1981) have also shown that the unperforated plastic bags helped preserve fruits and vegetables for short periods of storage. It is interesting to note that the half-life of folacin in Swiss chard appeared to be closely related to the loss of aesthetic qualities of the vegetable.

As shown in Fig. 2, when stored in open air in the produce box under refrigerated condition (4°C), Swiss chard retained 88% of the folacin over a 10 day period which is more time than would normally be expected from field to table. This agrees with Mullin et al. (1982) who found that storage at 4°C did not affect folacin content of Swiss chard adversely over a 14 day period. As the storage temperature increased, an increasing proportion of folacin was lost. For example, approximately 10, 30, and 43% of folacin loss occurred in 6 hr of storage at 21, 35, and 40°C, respectively (Fig. 2). Similar observatons on ascorbic acid retention in Swiss chard were made by earlier workers. Zepplin and Elvehjem (1944) reported 30 – 35% loss of vitamin C in Swiss chard stored at room temperature for 3 days, whereas no loss of the vitamin was observed at 8 – 10°C. McComb (1957) also found no loss of vitamin C in Swiss chard stored at 10 – 12°C for 4 days. Therefore, rapid cooling and storage at low temperature are beneficial for highly perishable vegetables in nutrient retention.

The loss of folacin in Swiss chard during storage at elevated temperatures also followed first order kinetics, with correlation coefficients greater than 0.98. The first order rate constants calculated from the slopes of the lines in Fig. 2 are 0.0123, 0.0625, and 0.0987/hr at 21, 35, and 40°C, respectively (Table 1). However, the order of the

Fig. 1–Changes in folacin content of Swiss chard under three storage conditions at room temperature (21°C).

Table 1—Effect of storage conditions on first-order rate constant and half-life for folacin degradation in Swiss chard

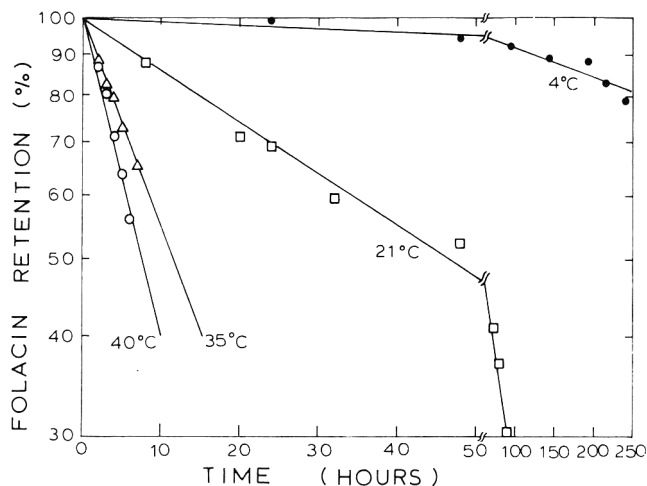| Storage condition	Correlation coefficient	Rate constant k (hr^{-1})	Half-life $t_{1/2}$ (hr)
In plastic bag at 21°C	0.998	0.00810	108
Under moist condition at 21°C	0.996	0.00896	77
In open air at: 4°C	0.925	0.00086	806
21°C	0.988	0.0123	56
35°C	0.992	0.0625	11
40°C	0.980	0.0987	7

Fig. 2—Folacin retention in Swiss chard stored in open air at different temperatures.
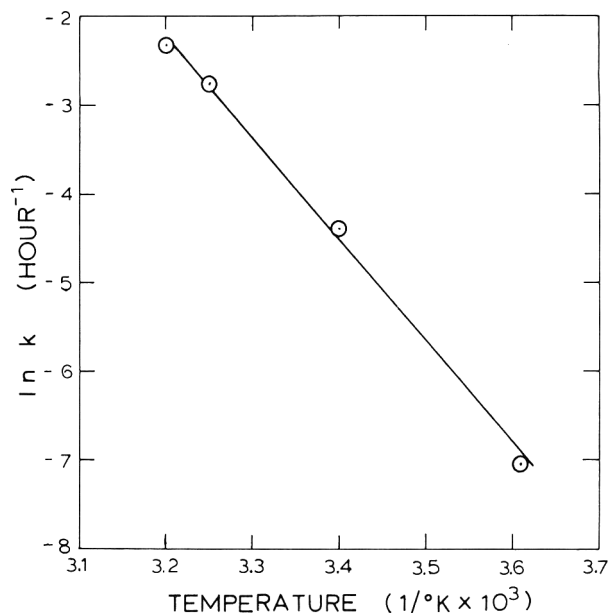

Fig. 3—Plot of rate constant (K) for folacin destruction and storage temperature of Swiss chard according to the Arrhenius equation.

reaction of folacin degradation at 4°C could not be ascertained since the data fit zero and first order kinetics equally well (r = 0.93). The first order rate constant calculated is 0.00086 and the half-life would be 33 days (Table 1).

The first order kinetic for the degradation reaction of the naturally occurring folates in Swiss chard during storage is in agreement with previous research. The thermal destruction reactions of synthetic folacin derivatives have been reported to be first order in solutions or in model food systems (Chen and Cooper, 1979; Mnkeni and Beverage, 1982, 1983), except under limited oxygen (Day and Gregory, 1983; Ruddick et al., 1980). Since the nature of the folacin derivatives in Swiss chard is unknown and the storage temperatures employed in the present study are much lower than the experimental temperatures for the thermal destruction studies reported in the literature, a comparison of the rate constants would not be meaningful.

The low values of the first order rate constants obtained in this study (Table 1) indicate that the folacin present in the Swiss chard is relatively stable under refrigerated and room storage conditions. One factor that could have contributed to the stability of folacin is the presence of a moderate amount of ascorbic acid in Swiss chard. Fresh chard contains 50 – 95 mg ascorbic acid/100g (Zepplin and Elvehjem, 1944) and a level of 80 – 140 mg ascorbic acid/100g was found to be sufficient to exert an antioxidative effect on the folate activity in Brussels sprouts during storage (Malin, 1977).

Conformation of the thermal inactivation data for folacin in fresh Swiss chard to the Arrhenius equation is shown graphically in Fig. 3 where the natural log of the rate constant is plotted against 1/T. The linearity of the line supports the use of the Arrhenius model for expressing the temperature dependence of the reaction rate constant for folacin. The Arrhenius activation energy (Ea) for the folacin degradation reaction in Swiss chard was calculated to be 24 kcal/mole which falls in the range of 20 – 30 kcal/mole, typical values of activation energy for vitamin destruction in foods (Saguy and Karel, 1980). In comparison, values of 9.5 kcal/mole (Chen and Cooper, 1979), 7.1 kcal/mole (Ruddick et al., 1980), and 17 – 19.8 kcal/mole in buffers and 7.85 – 10.8 kcal/mole in food model systems (Mnkeni and Beveridge, 1983) have been reported for 5-methyltetrahydrofolic acid, the only naturally occurring folate for which Ea has been published. The differences between the Ea values obtained in the present study and the literature values are attributable to the difference in the nature of the folates involved. Although the 5-methyl derivative of fola-

cin is the major form found in cabbage, lettuce and orange juice, the 5- or 10-formyl derivatives predominate in the soybean (Stokstad, 1979). Further work to identify the forms of folates present in the Swiss chard and other foods as well as to determine the Ea values for folate derivatives other than the 5-methyltetrahydrofolic acid is needed.

It appears that the rate of folacin loss in fresh Swiss chard during storage is low and the half-life of folacin coincides with the loss of the aesthetic qualities of the vegetable. Low temperature storage in plastic bags helped preserve the vitamin and visual qualities of fresh vegetables. However, the apparent differences in the activation energy for folacin destruction observed in Swiss chard and that occurring in food model systems suggest that more information is needed on the distribution of folacin derivatives in plant materials.

REFERENCES

Chanarin, I. 1981. Dietary deficiencies of vitamin B-12 and folic acid. In "Nutrition Problems in Modern Society," (Ed.) Howard, A.N. John Libby, London.
Chen, T.-S. and Cooper, R.G. 1979. Thermal destruction of folacin: effect of ascorbic acid, oxygen, and temperature. J. Food Sci. 44: 713.
Chen, T.-S., Lui, C.K.F., and Smith, C.H. 1983. Folacin content of tea. J. Amer. Diet. Assoc. 82: 627.
Day, B.P.E. and Gregory, J.F. 1983. Thermal stability of folic acid and 5-methyltetrahydrofolic acid in liquid model food systems. J. Food Sci. 48: 581.
Kenny, A. 1971. Storage of gooseberries in plastic bags. J. Food Technol. 6: 403.
Kirsch, A.K. and Chen, T.-S. 1984. Comparison of conjugase treatment procedures in the microbiological assay for food folacin. J. Food Sci. 49: 94.
McComb, C.L. 1957. Ascorbic acid oxidase activity of certain vegetables and changes in the dehydroascorbic acid during shelf-life. Food Res. 22: 448.
Maline, J.D. 1977. Total folate activity in Brussels sprouts: the effects of storage, processing, cooking and ascorbic acid content. J. Food Technol. 12: 623.
Mnkeni, A.P. and Beveridge, T. 1982. Thermal destruction of pteroylglutamic acid in buffer and model food systems. J. Food Sci. 47: 2038.
Mnkeni, A.P. and Beveridge, T. 1983. Thermal destruction of 5-methyltetrahydrofolic acid in buffer and model food systems. J Food Sci. 48: 595.
Mullin, W.J., Wood, D.F., and Howsam, S.G. 1982. Some factors affecting folacin content of spinach, Swiss chard, broccoli and Brussels sprouts. nutr. Rep. Int'l. 26: 7.

Chemical Composition and Nutritive Value
of Truffles of Saudi Arabia

W. N. SAWAYA, A. AL-SHALHAT, A. AL-SOGAIR, and M. AL-MOHAMMAD

──────────ABSTRACT──────────

The chemical composition and nutritional quality of truffles of Saudi Arabia, *Terfezia claveryi* and *Tirmania nivea*, were studied. Results showed 19.6 and 27.2% protein, 2.8 and 7.4% fat, 7.0 and 13.2% crude fiber, 4.6 and 5.4% ash and 1.8 and 5.1% ascorbic acid for *T. claveryi* and *T. nivea*, respectively. Both varieties contained high amounts of K and P and fair levels of Fe, Cu, Zn and Mn. All essential amino acids were present in fairly good amounts. Leucine and lysine were the first limiting amino acids in *T. claveryi* and valine in *T. nivea*. In-vitro protein digestibility (82.8 – 86.7%) was slightly lower than that of Animal Nutrition Research Council (ANRC) casein (90.0%) but the calculated protein efficiency ratio (2.1 – 2.49) was relatively close to the value of 2.5 for ANRC casein.

INTRODUCTION

TRUFFLE TUBERS are widespread around the world including various parts of the Middle East and North Africa. However, the most highly prized truffle is the Perigord truffle, *Tuber melanosporum* Vittad., that grows in Southern Europe. The Perigord truffle is valued for its aroma imparted to foods and is usually used as a condiment. In countries of the Middle East and North Africa, truffles are usually used in cooked dishes and have long been utilized by Arabs of the desert as substitutes for meat in their diet.

Unlike the European truffles which grow in forests in association with oak trees, the Saudi truffles are desert species found in the interior of Saudi Arabia excluding the deep sand and bare rocky regions. Records for the years 1966 – 1974 show that the interior regions of Saudi Arabia where such truffles grow have a mean temperature of 24.1°C and an annual average rainfall of 127 mm.

In Saudi Arabia, three types of truffles namely Gibaah, Kholeissi and Zubaidi are found, but two of these, i.e. Gibaah and Kholeissi (brown truffle) are different forms of the same species, *Terfezia claveryi* Chatin, (Terfeziaceae), while Zubaidi (white truffle) was identified as *Tirmania nivea* (Desf. Fr.)Trappe (Pezizaceae). The white truffle, *T. nivea*, produces the largest ascocarp (up to 1000g) and the brown truffle yields smaller ones (Khleissi, up to 300g and Gibaah up to 700g). Reports on the occurrence of these two species in the Middle East and North Africa are sporadic and at least one of these species was reported to be found in Kuwait, Iraq, Syria, Libya, Qatar, Morocco and Tunisia (Abdalla et al., 1979; Cook, 1984). Published information on their food values is still lacking and the limited data available in the literature was mostly reported from Kuwait, Iraq and Libya (Khalaf et al., 1970; Al-Delaimy, 1977; Hussein and Eid, 1980; Ahmed et al., 1981 and Al-Shabibi et al., 1982) and involves only one of these species, *Terfezia claveryi* Chatin. In Saudi Arabia only preliminary work relating to the proximate analysis and the contents of some mineral elements in these two truffle

species was done (Abdalla et al., 1979) but data is still lacking on the nutritional quality of the local truffles.

The purpose of this investigation was to determine the chemical composition and nutritional characteristics of the truffles growing in Saudi Arabia which may also be common to other countries in the area.

MATERIALS & METHODS

Collection of samples

Samples of two different species of truffles, *Terfezia claveryi* Chatin, including the brown Kholeissi and Gibaah varieties, and *Tirmania nivea*, including the white Zubaidi were purchased fresh from the local market and washed free of adhering soil. Three samples of each variety were purchased from different localities in Riyadh city and the samples (4 kg each) were thoroughly mixed. Representative samples from the composite lots were used for analysis. These truffles were those from Haffar Al-Batin area in the interior of Saudi Arabia about 500 kM north of Riyadh city. Since truffles sometimes are peeled due to the difficulty of removing all the dirt between the crevices, samples from each species collected were divided into two parts, peeled and unpeeled, and were blended separately by a Brabender chopper. Samples were withdrawn for moisture and vitamin C analysis and the remaining were immediately freeze-dried (Stokes, Model 902-1-8), ground and kept in air tight glass jars in the deep freeze (–40°C) for further analysis.

Chemical and nutritional analysis

Proximate analysis. Methods outlined in AOAC (1980) were used for the analysis of moisture (Method 7.003), crude fat (7.056), crude fiber (7.061), ash (7.009) and vitamin C (43.056). Crude protein (N × 6.25) was determined with a Kjeldahl (Protein/Nitrogen) analyser (Kjel-Foss Automatic Model 16210, A/S N. Foss Electric, Denmark) using the AOAC (1980) method (7.024).

Mineral element analysis. For the determination of mineral elements, (Na, K, Ca, Mg, P, Fe, Cu, Zn and Mn) the ash was dissolved in 5 mL 20% HCl. The final diluted solution for Ca and Mg contained 1% lanthanum to overcome interferences, especially by phosphates. All minerals except Na, K and P were determined with an atomic absorption spectrophotometer (Perkin Elmer, Model 603). Na and K were determined with a flame photometer (Beckman, Klina flame). P was determined spectrophotometrically using the procedure of Watanabe and Olsen (1965).

Amino acid analysis. Amino acid analysis was done by hydrolyzing under vacuum the freeze-dried samples containing 5 mg protein for 24 hr at 110°C (Moore and Stein, 1963). For tryptophan analysis, samples were hydrolyzed with 5N NaOH according to the method of Hugli and Moore (1972). The sulphur-containing amino acids were obtained using performic acid treatment and then hydrolyzing with 6N HCl (Moore, 1963). All hydrolysates were analyzed with a Beckman amino acid analyzer (Model 119CL).

In-vitro protein digestibility (IVPD) and calculated protein efficiency ratio (C-PER). IVPD was measured according to the method outlined by Satterlee et al. (1979) using a modification of the multienzyme automatic recording technique reported by Hsu et al. (1977). The C-PER was obtained by using data from IVPD and essential amino acid composition of the protein according to procedures outlined by Satterlee et al. (1979). Animal Nutrition Research Council (ANRC) casein was included for comparison.

Chemical score. The chemical scores were obtained by dividing the contents of the essential amino acids by the amounts of the same amino acids in the FAO/WHO reference protein (1973).

RESULTS & DISCUSSION

TABLE 1 shows the results of the chemical composition of the three truffle tubers. Results for the protein, fat, fiber and ash contents were generally higher than those of the most commonly consumed vegetables and tubers (Watt and Merrill, 1975; Pellet and Shadarevian, 1970). The white species of truffles *Tirmania nivea* (Desf. Fr.) Trappe (Zubaidi) contained considerably higher levels of protein, fat and fiber compared to the contents of these nutrients in the brown truffles, *Terfezia claveryi* Chatin. The contents of protein in the two Saudi Arabian brown truffles were lower than that in the truffle *Tuber melanosporum* (Delmas, 1980) and black truffles, *Terfezia claveryi* Chatin of Iraq (Khalaf et al., 1970; Al-Shabibi et al., 1982) but slightly higher than the protein content of 17.19% in the Libyan brown truffles, *Terfezia boudieri* Chatin. The contents of fat, ash and crude fiber of both the white and brown species of Saudi Arabian truffles were comparable to the contents of these nutrients in the Iraqi and Libyan truffles except that the Libyan truffles contained relatively higher levels of ash and the Saudi white truffles contained higher levels of crude fiber. The vitamin C contents of the Saudi truffles of both white and brown species were lower than the values reported in literature (Abdalla et al., 1979). The Gibaah variety contained a higher concentration of vitamins C than those of Kholeissi and Zubaidi varieties. The results for vitamin C are in close agreement with those reported for *Tuber melanosporum* (Delmas, 1980). Results of the present study showed considerable variations from those of Abdalla et al. (1979) who reported on a dry weight basis 68.25 and 69.96% protein, 0.36 and 0.37% fat and 32 and 107 mg% of vitamin C in the Zubaidi and Kholeissi truffles of Saudi Arabia, respectively. The moisture content of the three Saudi truffles (75.27 – 78.89%) was comparable with the values reported in the literature for other varieties of truffles (Khalaf et al., 1970; Abdalla et al., 1979; Al-Shabibi et al., 1982). Of the two Saudi Arabian brown varieties, Gibaah contained higher levels of protein, fat, ash and vitamin C even though both belonged to the same species *T. claveryi* Chatin, as identified by B.M.

Spooner (private communication) of the Kew Herbarium of the Royal Botanic Gardens, Surrey, England.

Peeling of truffles resulted in considerable decrease in the protein, fat, ash and ascorbic acid contents. The crude fiber content, on the other hand, tended to be higher in the peeled truffles. The reason for the higher fiber content in the peeled truffles can not be ascertained.

Mineral element contents

Table 2 shows the data on the mineral element contents of the truffles. The concentration of potassium was the highest in all the varieties followed by phosphorus, sodium, magnesium and calcium. In general, the levels of the macro elements in the three truffle samples were comparable to those found in commonly consumed tubers such as potatoes and other similar foods such as mushrooms. No major differences were observed in the levels of these macroelements in the different varieties investigated. Among the microelements, copper was present in higher concentration while iron and zinc were present in fair amounts when compared to their daily needs (Food and Nutrition Board, 1980). Gibaah variety showed a higher concentration of iron while the white truffle, Zubaidi, showed a considerably higher concentration of copper compared to the other two varieties. In comparison with other truffles, the local varieties contained comparable amounts of potassium to those of Libyan and Kuwaiti truffles (Ahmed et al., 1981; Hussein and Eid, 1980) and *T. melanosporum* (Delmas, 1980). The calcium content was similar to that of the Libyan truffles but considerably lower than that of *T. melanosporum* truffles and Kuwaiti truffles which also contained higher levels of magnesium. The local truffles were also lower in the levels of iron, zinc, copper and manganese when compared to the levels of these microelements in Libyan and Kuwaiti truffles. However, the iron content was similar to that of *T. melanosporum* truffles and the copper content was in agreement with that of the Italian piedmantese truffles (Barbero, 1974). With respect to the levels of these mineral elements in the same species of Saudi truffles reported by Abdalla et al. (1979), the contents of potas-

Table 1—Chemical composition of truffles

		Moisture %	Protein* %	Fat* %	Crude Fiber* %	Ash* %	Ascorbic Acid* (mg/100 g)
Whole							
Unpeeled:	Gibaah (B)**	75.44	24.96 ± 0.04	4.20 ± 0.31	7.02 ± 0.22	6.39 ± 0.36	5.10 ± 0
	Kholeissi (B)**	78.89	19.59 ± 0.04	2.81 ± 0.03	7.85 ± 0.13	4.64 ± 0.28	1.82 ± 0
	Zubaidi (W)**	75.27	27.18 ± 0.04	7.42 ± 0.41	13.02 ± 0.02	5.40 ± 0.08	1.56 ± 0
Peeled:	Gibaah	79.39	20.26 ± 0.05	3.43 ± 0.53	10.48 ± 0.16	4.46 ± 0.19	1.42 ± 0
	Kholeissi	78.29	19.65 ± 0.05	3.43 ± 0.09	7.81 ± 0.15	4.33 ± 0.38	0.70 ± 0
	Zubaidi	75.21	25.49 ± 0.05	7.19 ± 0.34	14.89 ± 0.28	5.00 ± 0.52	1.00 ± 0

* Values are averages of 4 determinations ± standard deviation on dry weight basis
** B = Brown species, W = White species

Table 2—Mineral composition of truffles[a]

Truffles	Ca	Mg	P	Na	K	Fe	Mn	Cu	Zn
Unpeeled:									
Gibaah	129 ± 29.1	104 ± 6.9	756 ± 35.3	199 ± 23.5	1730 ± 60.4	10.68 ± 1.03	0.48 ± 0.06	1.69 ± 0.20	5.10 ± 0.11
Kholeissi	67 ± 30.6	82 ± 2.7	506 ± 11.3	189 ± 24.3	1408 ± 62.2	4.81 ± 0.23	0.41 ± 0.05	2.30 ± 0.37	4.33 ± 0.35
Zubaidi	62 ± 16.3	101 ± 1.0	644 ± 27.3	110 ± 9.5	1734 ± 8.7	4.35 ± 0.20	0.49 ± 0.03	11.54 ± 0.48	5.04 ± 0.19
Peeled:									
Gibaah	41 ± 2.6	74 ± 2.3	653 ± 7.6	184 ± 13.7	1548 ± 31.3	1.82 ± 0.26	0.21 ± 0.02	1.33 ± 0.24	3.69 ± 0.24
Kholeissi	34 ± 8.2	73 ± 3.4	513 ± 23.1	142 ± 16.9	1393 ± 29.7	1.75 ± 0.45	0.22 ± 0.10	1.95 ± 0.20	3.71 ± 0.30
Zubaidi	38 ± 5.9	97 ± 6.9	623 ± 23.9	199 ± 20.0	1602 ± 56.6	2.31 ± 0.43	0.44 ± 0.09	8.74 ± 0.32	4.53 ± 0.22

[a] Average values of 4 determinations ± standard deviations expressed as mg/100 g dry weight

Table 3—Amino acid composition of truffles (g/100g protein)[a]

	Unpeeled Truffles			Peeled Truffles			FAO/WHO (1973) Ref. protein pattern
	Gibaah	Kholeissi	Zubaidi	Gibaah	Kholeissi	Zubaidi	
Aspartic acid	8.04 ± 0.18	8.25 ± 0.35	8.83 ± 0.07	8.24 ± 0.07	7.81 ± 0.28	8.57 ± 0.40	—
Threonine	5.38 ± 0.14	4.09 ± 0.06	7.81 ± 0.06	5.24 ± 0.11	4.00 ± 0.14	7.19 ± 0.40	4.0
Serine	3.96 ± 0.03	3.52 ± 0.01	4.29 ± 0.08	4.19 ± 0.04	3.38 ± 0.23	4.56 ± 0.16	—
Glutamic acid	13.37 ± 0.28	14.78 ± 0.27	14.37 ± 0.20	17.98 ± 0.30	15.19 ± 0.50	14.79 ± 0.69	—
Proline	5.03 ± 0.11	4.01 ± 0.40	4.95 ± 0.06	5.67 ± 0.57	3.97 ± 0.22	5.94 ± 0.55	—
Glycine	3.58 ± 0.07	3.64 ± 0.01	4.33 ± 0.06	3.90 ± 0.03	3.51 ± 0.09	4.35 ± 0.20	—
Alanine	6.42 ± 0.13	5.01 ± 0.03	6.46 ± 0.06	6.79 ± 0.09	4.90 ± 0.15	6.43 ± 0.20	—
Valine	3.96 ± 0.07	3.71 ± 0.02	4.65 ± 0.13	3.95 ± 0.03	3.74 ± 0.33	4.77 ± 0.15	5.0
Methionine (M)	4.23 ± 0.05	3.14 ± 0.09	3.22 ± 0.26	4.01 ± 0.12	3.00 ± 0.17	3.80 ± 0.19	—
Cystine (C)	1.62 ± 0.20	1.26 ± 0.02	1.30 ± 0.06	1.78 ± 0.29	1.14 ± 0.47	1.44 ± 0.30	—
M + C	5.85 ± 0.25	4.40 ± 0.21	4.52 ± 0.32	5.79 ± 0.42	4.14 ± 0.64	5.24 ± 0.48	3.5
Isoleucine	3.69 ± 0.05	3.54 ± 0.03	4.42 ± 0.11	3.74 ± 0.11	3.36 ± 0.09	4.39 ± 0.19	4.0
Leucine	5.23 ± 0.09	5.28 ± 0.10	5.50 ± 0.10	5.65 ± 0.03	4.94 ± 0.11	5.14 ± 0.18	7.0
Tyrosine (T)	2.90 ± 0.02	3.42 ± 0.13	3.09 ± 0.08	2.76 ± 0.11	3.11 ± 0.05	3.11 ± 0.37	—
Phenylalanine (P)	2.97 ± 0.04	3.17 ± 0.06	3.37 ± 0.05	3.11 ± 0.02	3.04 ± 0.04	3.32 ± 0.03	—
T + P	5.87 ± 0.06	6.59 ± 0.19	6.46 ± 0.12	5.87 ± 0.13	6.15 ± 0.18	6.43 ± 0.40	6.0
Lysine	4.13 ± 0.05	5.49 ± 0.10	5.70 ± 0.29	5.22 ± 0.03	5.40 ± 0.28	4.93 ± 0.19	5.5
Histidine	1.45 ± 0.02	2.09 ± 0.03	2.18 ± 0.17	2.00 ± 0.03	2.18 ± 0.02	1.70 ± 0.07	—
Arginine	3.20 ± 0.05	7.95 ± 0.16	4.46 ± 0.16	4.46 ± 0.03	8.37 ± 0.19	3.52 ± 0.17	—
Tryptophan	1.20 ± 0.03	1.23 ± 0.0	1.44 ± 0.06	1.27 ± 0.09	1.20 ± 0.15	1.30 ± 0.03	1.0

[a] Average values of 4 determinations ± Standard deviations

sium and magnesium reported in the present study were relatively comparable to the values (dry basis) of 1610 mg and 1826 mg/100g for potassium and magnesium, respectively. However, the phosphorus content was several times higher than that reported by the above authors (141 – 214 mg/100g) while the iron, Zn and Mn contents were lower than the iron (58.1 – 63.6 mg/100g) and Zn (5.5 – 16.2 mg/100g) and Mn (3.6 – 6.9 mg/100g) contents reported by Abdalla et al. (1979).

Peeling of truffles showed a reduction in the levels of all mineral element contents which was more pronounced in case of calcium, iron and to a lesser extent manganese and zinc.

Amino acid composition

Data on the amino acid composition is presented in Table 3. Eighteen amino acids including tryptophan were determined. All the essential amino acids were present in fairly good amounts. Of the sulphur-containing amino acids (methionine + cystine), tryptophan and lysine, which are usually limiting in many foods of plant origin, the former two were present in amounts exceeding the FAO/WHO (1973) reference protein pattern while lysine along with leucine were the limiting amino acids in the Gibaah variety and constituted approximately 75% of the FAO/WHO (1973) reference protein requirement. Leucine was limiting in the Gibaah and Zubaidi variety while valine was limiting in the Kholeissi. In general, the amino acid profiles of the truffles indicated a good nutritional quality of the truffle proteins with chemical scores (Table 4) of unpeeled truffles ranging between 74 – 79 for the three varieties. No major differences were observed in the amino acid profiles of the three truffle varieties except arginine, whose concentration was twice as much in Kholeissi variety as that in the other two varieties. Furthermore, peeling did not affect the amino acid composition of the truffles to any significant extent. When these results were compared with those reported in the literature, the amino acid pattern of Saudi truffles varied considerably from those of Iraqi truffles, *T. claveryi* and *Terfezia hafizi* in which sulphur amino acids (methionine + cystine) were the most deficient essential amino acids (Al-Delaimy, 1977) and also from the Libyan truffles, *T. boudieri*, in which lysine was reported to

Table 4—Chemical score, IVPD and C-PER of truffles

Truffles	Chemical score	First limiting amino acid	IVPD[a] %	C-PER[a]
Unpeeled				
Gibbah	75	lysine leucine	84.6 ± 0.19	2.09 ± 0.0
Kholeissi	74	valine	82.8 ± 0.23	2.10 ± 0.0
Zubaidi	79	leucine	86.7 ± 1.12	2.49 ± 0.0
Peeled				
Gibaah	79	valine	87.3 ± 3.14	2.35 ± 0.1
Kholeissi	71	leucine	83.6 ± 0.53	2.06 ± 0.32
Zubaidi	73	leucine	86.6 ± 2.71	2.35 ± 0.06
ANRC-casein[b]	—		90.0	2.50

[a] Average values of 4 determinations ± standard deviations
[b] Animal Nutrition Research Council casein

be the first limiting essential amino acid (Ahmad et al., 1981).

IVPD and C-PER

The results of IVPD and C-PER (Table 4) showed that the white truffle, *Tirmania nivea* (Zubaidi) had a higher IVPD and C-PER value and therefore, had a better nutritional quality over the two brown varieites (Gibaah and Kholeissi). The nutritional quality of the white truffles was comparable to that of the ANRC-casein. The IVPD values of the three truffles did not show any major difference but the C-PER values of the two brown varieties were relatively lower than that of the white Zubaidi variety. Peeling of truffles showed a slight increase in the IVPD values but did not indicate any consistent trend on the C-PER.

The results of this study showed that considerable variation may be found in the chemical composition of truffles which might be due to differences in species and/or environment under which they grow. Saudi truffles like other truffles reported in the literature are of good nutritional quality.

REFERENCES

Abdalla, N.Y., Hassan, Y.M., Mossa, G.S., and El-Yahia, M.A. 1979. Studies on the nutritive value of Saudi truffles and the possibility of their preservation by canning. Proc. 2nd Arab Conf. Fd. Sci. Technol. p. 369.

Ahmed, A.A., Mohamed, M.A., and Hami, M.A. 1981. Libyan truffles "Terfezia boudieri Chatin." Chemical composition and toxicity. J. Food Sci. 46(3): 927.

Al-Delaimy, K.S. 1977. Protein and amino acid composition of truffle. Can. Inst. Food Sci. Tech. J. 10(3): 221.

Al-Shabibi, M.M.A., Toma, S.J., and Haddad, B.A. 1982. Studies on Iraqi Truffles. 1. Proximate analysis and characterization of lipids. Can. Inst. Food Sci. Tech. J. 15(3): 200.

AOAC. 1980. "Official Methods of Analyses," 13th ed. Association of Official Analytical Chemists, Washington, DC.

Barbero, L. 1974. Copper content of white piedmontese truffles. Industrie Alimentari 13(12): 77.

Cook, A.A. 1984. Truffles — An Overview. Regional Agriculture and Water Research Center, Miscellaneous publication. Ministry of Agriculture and Water, Kingdom of Saudi Arabia, Riyadh.

Delmas, J. 1980. The truffle and its cultivation. Miscellaneous report translated from French for the US Department of Agriculture and National Science Foundation by Al-Ahram Center for Scientific Translation.

FAO/WHO. 1973. Energy and protein requirements. FAO Nutritional Meeting Report Series No. 52, WHO Technical report series No. 522. Food and Agriculture Organization, Rome.

Food and Nutrition Board. 1980. Recommended Dietary Allowances. 9th ed. National Academy of Sciences, Washington, DC.

Hugli, T.E. and Moore, S. 1972. Determination of the tryptophan content of proteins by ion exchange chromatography of alkaline hydrolyzates. J. Biol. Chem. 247: 2828.

Hussein, M.A. and Eid, N.M. 1980. Nutrients components of truffles. Nahrung 24(8): 811.

Hsu, H.W., Vavak, D.L., Saterlee, L.D. and Miller, G.A. 1977. Multienzyme technique for estimating protein digestibility. J. Food Sci. 42: 1269.

Khalaf, S., Al-Delaimy, K.S., Sagida, H., and Abu Gharib, A. 1970. Storage, spoilage and proximate composition of Iraqui truffles. Beit. Trop. Subtrop. Landwrit. Tropenveternmedizin 8: 77.

Moore, S. 1963. On the determination of cystine as cysteic acid. J. Biol. Chem. 238: 235.

Moore, S. and Stein, W.H. 1963. Chromatographic determination of amino acids by the use of automatic recording equipment. Methods in Enzymology 6: 819.

Pellett, P.L. and Shadarevian, S. 1970. "Food composition tables for use in the Middle East", 2nd ed. American University of Beirut, Beirut, Lebanon.

Satterlee, L.D., Marshall, H.F. and Tennyson, J.M. 1979. Measuring protein quality. J. Amer. Oil Chem. Soc. 56: 103.

Watanabe, F.S. and Olsen, S. 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Sci. Soc. Amer. Proc. 29: 677.

Watt, B.K. and Merrill, A.L. 1975. "Composition of Foods" (Agricultural Handbook No. 8). Agricultural Research; U.S. Dept. of Agriculture, Washington, DC.

HYDRATION OF COWPEA MEAL . . . From page 446

Eastman, P. 1980. An end to pounding. International Development Research Center, Bull. IDRC-152e, p. 1.

Fery, R.L. 1981. Cowpea production in the United States. HortScience 16(4): 474.

Hall, A. E. 1984. Personal communication. Univ. California, Riverside, CA.

Helwig, J.T. and Council, K.A. (Ed.), 1979. "SAS User's Guide." SAS Institute Inc., Cary, NC.

McWatters, K.H. 1983. Compositional, physical, and sensory characteristics of akara processed from cowpea paste and Nigerian cowpea flour. Cereal Chem. 60: 333.

McWatters, K.H. and Brantley, B.B. 1982. Characteristics of akara prepared from cowpea paste and meal. Food Technol. 36(1): 66.

Onayemi, O. and Potter, N.N. 1976. Cowpea powders dried with methionine: preparation, storage stability, organoleptic properties, nutritional quality. J. Food Sci. 41: 48.

Reichert, R.D., Lorer, E.F., and Youngs, C.G. 1979. Village-scale mechanical dehulling of cowpeas. Cereal Chem. 56: 181.

Reichert, R.D. and Youngs, C.G. 1976. Dehulling cereal grains and grain legumes for developing countries. 1. Quantitative comparison between attrition- and abrasive-type mills. Cereal Chem. 53: 829.

Williams, C.E. 1980. Cowpea flour innovation in a traditional dish and its acceptability in selected areas of Oyo division. Ph.D. dissertation. Univ. of Ibadan, Nigeria.

FOLACIN STABILITY IN SWISS CHARD . . . From page 449

Olsen, O.E., Burris, R.H., and Elvehjem, C.A. 1947. A preliminary report of the folic acid content of certain foods. J. Amer. Diet. Assoc. 23: 200.

Peterkin, B.B., Patterson, P.C., Blum, A.J., and Kerr, R.L. 1981. Changes in dietary patterns: One approach to meeting standards. J. Amer. Diet. Assoc. 78: 453.

Ruddick, J.E., Vanderstoep, J., and Richards, J.F. 1980. Kinetics of thermal degradation of methyltetrahydrofolic acid. J. Food Sci. 45: 1019.

Saguy, I., and Karel, M. 1980. Modeling of quality deterioration during food processing and storage. Food Technol. 34(2): 78.

Stokstad, E.L.R. 1979. Overview of folic acid derivatives in animal tissues. In "Folic Acid in Neuology, Psychiatry, and Internal Medicine," (Eds.) Botez, M.I. and Reynolds, E.H. Raven Press, New York.

Umiecka, L. 1981. The effect of different factors on the stability of carrots for preparing in PE bags and their storage. Acta Hort. 116: 121.

Zepplin, M. and Elvehjem, C.A. 1944. Effect of refrigeration on retention of ascorbic acid in vegetables. food Res. 9: 100.

Moisture Sorption of Dry Bakery Products by Inverse Gas Chromatography

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─────────ABSTRACT─────────

Inverse gas chromatography was used to study moisture sorption properties of two bakery products as a function of temperature and food structure. Crackers (high in fat, low in sugar) were found to be more hygroscopic than sweet biscuits. Sorption isotherms at different temperatures were determined to obtain thermodynamic parameters of sorption. Enthalpy and entropy functions as well as Zimm-Lundberg clustering analysis suggested different sorption mechanisms in the two systems. Active site binding mechanism was manifested in the cracker system by highly negative enthalpy and entropy changes at low moisture contents. Water-water interactions were more favored in the sweet biscuit system at the same free energy level. Addition of crystalline sucrose decreased hygroscopicity of the cracker system and increased formation of water-water interactions.

INTRODUCTION

BISCUITS AND COOKIES are dry bakery products whose major ingredients are wheat flour, sugar, and shortening. Cracker formulae include very little or no sucrose. The cracker structure is basically that of a gluten network in which are embedded the starch grains (Flint and Moss, 1970). In short sweet biscuits the structure is made up mainly by fat and sugar bridges, and no continuous protein network is present (Francis and Grove, 1962). Part of the sugar stays in crystalline state through the baking process (Kissell et al., 1973; Pomeranz, 1973) or may recrystallize after baking. Fat is present in the cracker system as globules and as continuous layer enveloping the protein-starch network. In the sweet biscuit system, starch and protein particles are sealed within a thin film of fat (Flint and Moss, 1970).

An inherent quality characteristic of dry bakery products is their crispness. They lose quality during storage mainly through two deterioration processes: fat autoxidation causing rancidity and moisture pickup leading to loss of crispness (Helen and Aaltio, 1978). Moisture effects on dry bakery products and snacks have been studied widely from the crispness point of view (Katz and Labuza, 1981), but information is lacking on the thermodynamics and mechanism of water sorption in these food systems.

Moisture sorption isotherm of a food relates the moisture uptake by the food solids as a function of the equilibrium vapor pressure of water surrounding the food at the same temperature. In thermodynamic terms, sorption isotherms are essentially the relation of total free energy change to the amount sorbed (Young and Crowell, 1962). From the practical point of view, moisture sorption isotherms are a necessary part of information needed by a food manufacturer for predicting the shelf life of moisture sensitive foods, such as dry bakery products. They are part of the data required for calculating the required package

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barrier properties for different storage and distribution conditions.

Inverse Gas Chromatography (IGC) is a rapid, effective system for studying the thermodynamic properties of a solid used for the stationary phase in relation to a mobile gas phase containing selected solutes such as water. It has been used successfully in this laboratory to generate data for determination of water vapor sorption isotherms of a single, homogeneous solid food ingredient, such as sucrose, glucose, and different starches (Smith et al., 1981; Smith, 1982). A recent review of IGC theory also covers food applications (Gilbert, 1984). In this research the IGC technique was applied to investigate moisture sorption in complex, heterogeneous dry bakery product systems. The objectives of this work were: (1) to study the effects of temperature and food structure on the moisture sorption of two compositionally different dry bakery product systems: a cracker and a sweet biscuit; (2) to determine from the thermodynamic parameters of moisture sorption, the nature of the water/food component and water/water interactions.

MATERIALS & METHODS

PLAIN UNSALTED CRACKERS, plain uncoated sweet biscuits, both manufactured by Nabisco Inc., (East Hanover, NJ), and granulated pure cane sugar, manufactured by The National Sugar Refining Co., (NY), were used as experimental material.

Conventional static method isotherms were determined by equilibrium with a range of saturated salt solutions (Rockland, 1960; Helen, 1983).

Crackers and biscuits were ground in a mortar and sieved in a mechanical sieving instrument. Particles passed through the 35 mesh/sieve and left on the 49 mesh/sieve were mixed with inert diatomaceous support material (Supelcoport 60/80) at 1 – 2% (w/w) loading level. A weighed amount of that mixture was packed in a 50 cm by 6.3 cm (1/4 in.) (o.d.). The amount of food in the column was optimized so that the diffusional effects causing peak broadening were minimized. However, loadings were high enough to separate the retention times of the noninteractive agent (air) and the water vapor.

Unpublished studies (Apostolopolous and Gilbert, 1984) have shown that particle size of ground hydrophilic foods has only a minor effect on sorption isotherms determined by IGC. The fibrous structure of these baked goods also aided in eliminating most of the diffusional and other kinetic factors limiting equilibrium attainment which contribute to peak broadening.

Each data set was duplicated with different columns with the same type of loading with no significant differences relative to sample type when appropriate care was taken to condition the stationary phase with dry helium carrier gas to an initial steady response equal to the blank helium. The column was installed in the oven of a Varian 200 dual column gas chromatograph equipped with a thermal conductivity detector and conditioned for 24 hr with carrier gas before starting the experiment.

In the IGC experiments distilled water probes of 0.1–5.0 μL were pulsed through the column by dry carrier gas. Triplicate injections were made. Sorption isotherms were determined from the chromatograms using the method presented by Kiselev and Yashin (1969). Peak areas were measured by a planimeter for substitution in the equations for pressure and amount absorbed (Coelho et al., 1979). Sorption experiments were run at four temperatures: 25°, 30°, 35°, and 40°C. The operational GC conditions were: column temperature: 25°, 30°, 35°, 40°C; injector temperature: 150°C;

detector temperature: 155°C; filament current: 150 mA; carrier gas: helium, dried by a moisture trap before columns; carrier gas flow rate: 30 cc/min.; recorder chart speed: 0.85 cm/min.

Statistical analyses (ANOVA) were performed on isothermal data by comparing the slopes of linearized sorption isothermal data by two model equations from the Hewlett Packard Statistical Package.

$$m = Ae^{Bp} \text{ or } m = C = Dp = Ep^2$$

where m is moisture uptake and p is the respective water vapor pressure obtained from the IGC data as discussed above.

A, B, C, D, E, are estimated parameters.

All data gave R^2 values ranging from 0.97 – 0.99.

Clustering analysis

In order to gain more insight into the mechanism of water sorption, the Zimm-Lundberg clustering analysis was done on the sorption data. This approach includes a direct measurement of nonrandom mixing in a two-component system without using any preconceived model. The cluster theory (Zimm, 1953; Zimm and Lundberg, 1956) defines a function measuring the tendency of the sorbed molecules to cluster and provides a means to calculate the average number of sorbate molecules in a cluster. Clustering analysis has been used to explain sorption processes in different sorbent-sorbate systems (Rogers et al., 1959; Starkweather, 1963; 1975; Lieberman et al., 1972; Coelho et al., 1979).
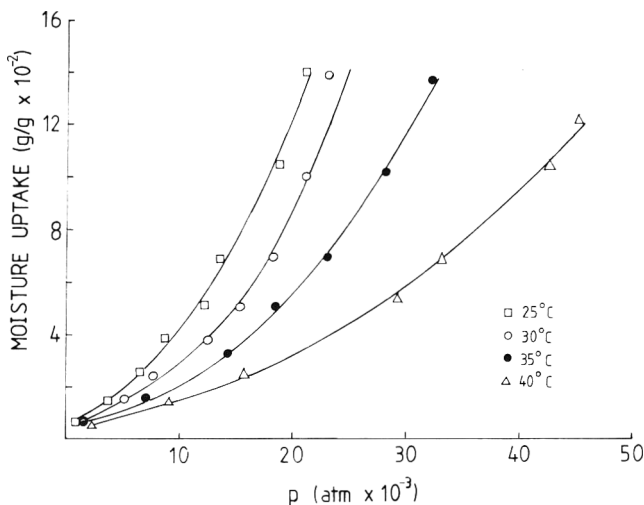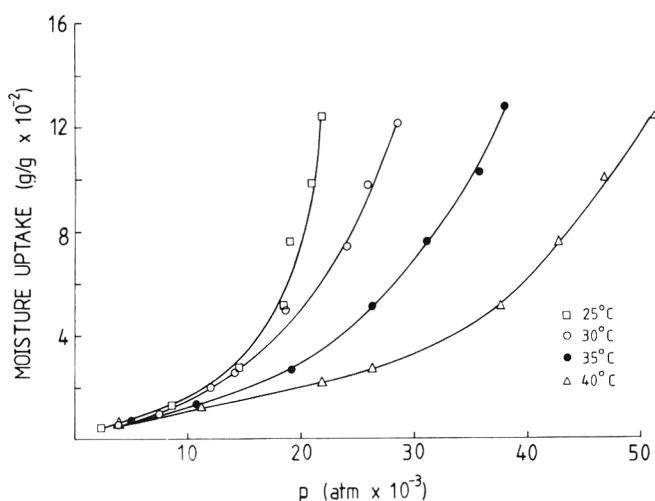
RESULTS & DISCUSSION

Sorption isotherms

Sorption isotherms of the cracker and the sweet biscuit systems at 25°, 30°, 35°, and 40° are shown in Fig. 1 and 2, where moisture uptake is plotted as a function of water vapor pressure. The sorption data indicate the expected temperature effect in both systems i.e., the higher the temperature the less moisture is absorbed at a given vapor pressure. Sorption isotherms of similar shape using static methods have been reported for biscuits and cookies at room temperature (Nemitz, 1968; Zabik et al., 1979). The shape of the isotherms suggests a very heterogeneous binding surface, characteristic of a complex food system, such as a dry bakery product. The isotherms are convex toward the axis. This suggests that the adsorption was cooperative in nature: the more molecules already adsorbed, the easier it was for further molecules to become adsorbed. The main cause for the shape of the isotherms, which reflects the presence of hydrophobic surfaces, was probably the fat component which, during the baking, will cover a major part of the active sites of flour. In the sweet biscuit structure at least part of the sugar was in the crystalline state thus flattening the isotherm at low water vapor pressures. Sorption isotherms obtained by the static method and IGC measurements were in reasonable agreement (Fig. 2) validating the use of the more rapid, continuous and sensitive IGC procedure for food particles.

The sorption isotherms of the cracker and the sweet biscuit at 30°C are plotted in the same graph for comparison (Fig. 3). The cracker absorbed significantly more moisture than the sweet biscuit at the same water vapor pressure. Also the cracker isotherm was steeper than the sweet biscuit isotherm at low water vapor pressures.

Mixtures of cracker:crystalline sucrose was prepared at three levels (100:0, 80:20, and 50:50, w/w), by mixing ground cracker particles with sucrose crystals of the same average particle size (35 – 45 mesh). Sorption isotherms were determined for each mixture at the four temperatuers and from the temperature dependence of the isotherms the thermodynamic parameters of sorption were calculated.

Fig. 1—Sorption isotherms of cracker at different temperatures.
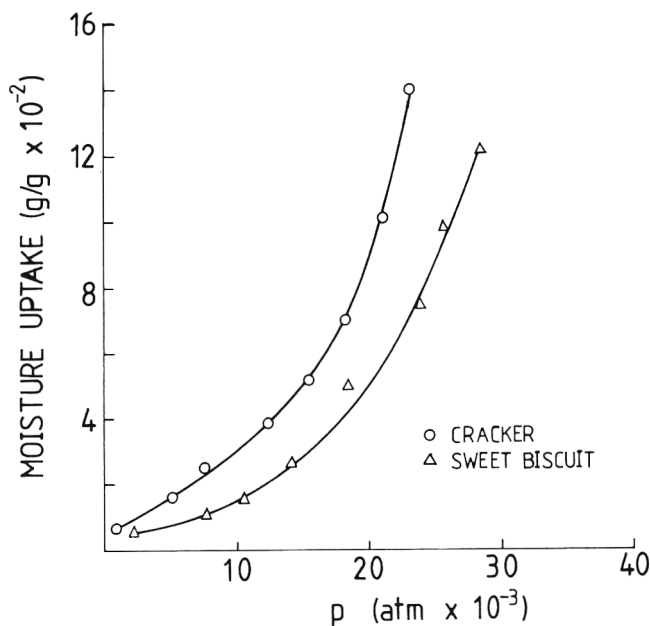

Fig. 2—Sorption isotherms of sweet biscuit at different temperatures.

Fig. 3—Sorption isotherm of cracker and sweet biscuit at 30°C.

Thermodyamics of moisture sorption

The total free energy change ΔG required to transfer water molecules from the vapor state to the food surface is a quantitative measure of the affinity of the food for the water. The excess ΔG_s is the key thermodynamic parameter in evaluating the interaction propensity of a food system with water. The excess free energy change, ΔG_s, at temperature T was calculated from the following equation:

$$\Delta G_s = RT \ln p/p_o$$

where: R is the universal gas constant; T is absolute temperature; p/p_o is the relative water vapor pressure @T with pure water as the standard.

In Fig. 4 the free excess energy changes of the two systems are presented as a function of moisture uptake. The negative ΔG_s values indicate that moisture sorption on food solids is a spontaneous process; the lower the moisture content the stronger the tendency towards sorption. The ΔG_s values of the cracker system were significantly more negative at all moisture levels than those of the sweet biscuit system in agreement with higher potential of the cracker for moisture sorption than the sweet biscuit.
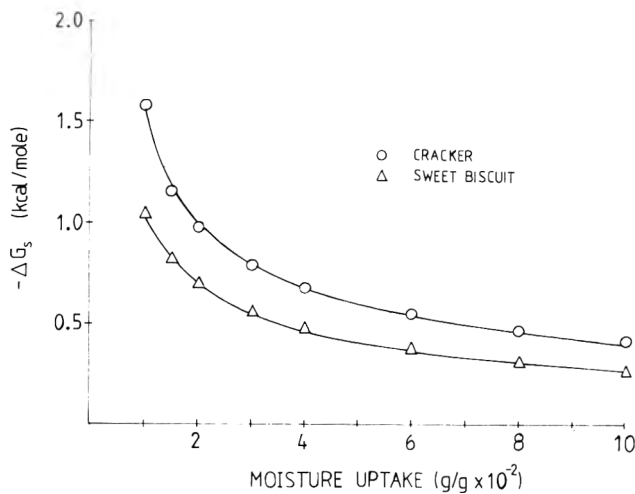
The differential or isosteric enthalpy of sorption, ΔH_s, is a molar quantity directly related to the energy of interaction between sorbating water molecules and the sorption sites in the food. This energy of interaction can suggest the sorption mechanism involved. As Iglesias and Chirife (1976) noted, direct calorimetric measurement of sorption enthalpies in foods were difficult because of the small amount of heat evolved. In this study, the ΔH_s values were calculated from the temperature dependence of the computer fitted isotherms using the Clausius-Clapeyron equation as described by Coelho et al. (1979). This plot of $\ln P_1$ vs $1/t$ gives a slope equal to $\Delta H_s/R$.

Once ΔG_s and ΔH_s are known the respective entropy change of sorption, ΔS_s, can be calculated from the Gibb's equation:

$$\Delta S_s = \frac{\Delta H_s - \Delta G_s}{T}$$

The term ΔS_s describes the change in the randomness or disorder of the food-water system during the sorption process.

The calculated enthalpy and entropy changes of sorption in the two systems at 30°C are presented as a function of moisture uptake in Fig. 5 and 6. The shapes of the enthalpy and entropy functions are closely similar which is in agreement with behavior found by Bettelheim and Vol-

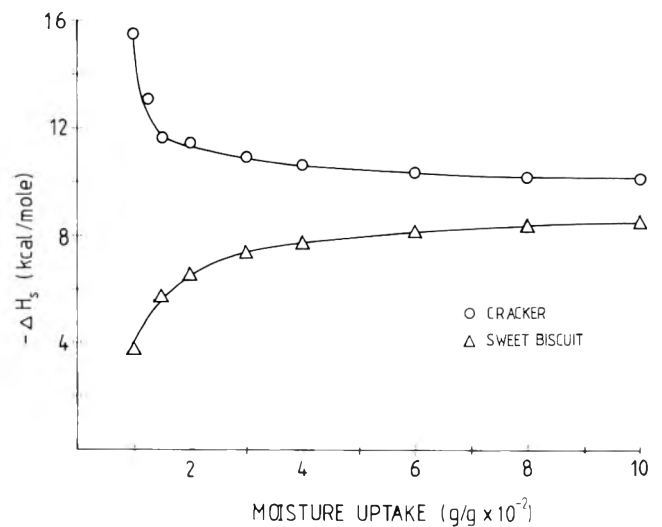Fig. 4—Free energy of sorption as a function of moisture uptake at 30°.

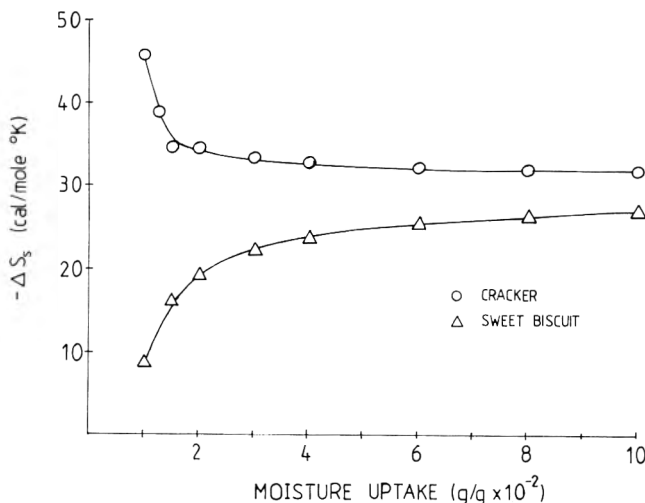Fig. 5—Enthalpy of sorption as a function of moisture uptake.

Fig. 6—Entropy of sorption as a function of moisture uptake at 30°C.
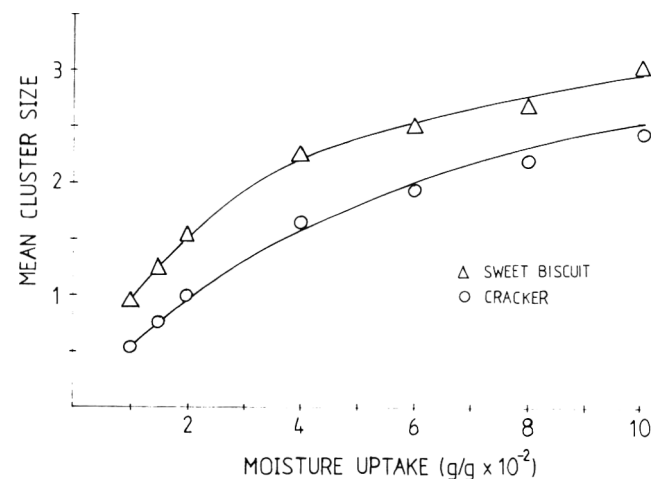

Fig. 7—Mean cluster size as a function of moisture uptake at 30°C.

man (1957), and Everett (1950) suggesting that a high degree of H/S compensation occurred. The enthalpy and entropy values of the cracker-water system showed characteristics typical of an active site binding process. At very low moisture contents the most active (energetically most favorable) sites became occupied by water molecules as reflected by the high negative ΔH_s (-15.4 Kcal/mole) and ΔS_s (-45.4 cal/mol) values. These are consistent with those reported for starch (Chung and Pfost, 1967; Smith, 1982) and proteins (Kuntz and Kausman, 1974). Less active sites were occupied giving rise to lower interaction energy, and at about $4 - 5\%$ moisture content water-water interactions started to become prevalent as indicated by ΔH_s values close to ΔH of vaporization of pure water (about -10.4 kcal/mole). The observed net enthalpy change of -5 kcal/mole at 1% moisture level probably reflects hydrogen bonding between the first water molecules and active polar groups in the protein-starch matrix.

The enthalpy and entropy changes of the sweet biscuit system showed the opposite behavior to that in the cracker system. The H_s and S_s functions changed from less negative values at very low moisture content to more negative values as more moisture was gained by the system. This suggests that some structural changes, besides the physical adsorption of water vapor on the food solids, takes place in the sweet biscuit matrix during the sorption process. Swelling

of the structure and incipient local dissolution of sugar surfaces are possible explanations for the observed behavior of the enthalpy and entropy functions in the sweet biscuit system (Bettelheim et al., 1970; Iglesias et al., 1976).

The opposite behavior of both the enthalpy and entropy functions in the two systems suggests that different sorption mechanisms were involved. The thermodynamic data clearly reflect that in the cracker system at low moisture levels the sorption took place by an active site, Freundlich-type binding mechanism changing gradually into water-water interactions with formation of water clusters. In the sweet biscuit system the active site binding mechanism was masked by structural changes in the food matrix giving rise to a positive entropy production.

Clustering analysis

The mean cluster size in the two systems at $30°C$ is plotted as a function of moisture uptake in Fig. 7. The data show that the mean cluster size was greater in the sweet biscuit system at all moisture levels. This indicates that in the cracker system there was a greater ratio of water-food site interactions than in the sweet biscuit system where water-water interactions were more favored.

Crystalline sucrose in the cracker system

The differences in the sorption behavior of the two dry bakery product systems were considered to be due to the basic difference in their structures. Since the cracker formula included no sucrose, addition of crystalline sucrose was made to the cracker system to determine its affect on the sorption characteristics.

Isotherms and thermodynamic parameters at $30°C$ are presented in Fig. 8, 9, 10, and Table 1. The more crystalline sucrose added to the cracker the closer the isotherm resembled that of the sweet biscuit (Fig. 8). Crystalline sucrose addition decreased the average hygroscopicity of the cracker system as a result of the dilution of the macromolecular active sites capable of binding water molecules. This observation is consistent with the results of El-Warraky et al. (1980) who also reported that increasing sugar concentration in biscuits decreased moisture sorption.

Thermodynamic support for the above conclusion was provided by the ΔG_s values which became less negative and approached the respective ΔG_s values of the sweet biscuit system with increasing sucrose concentration, thus
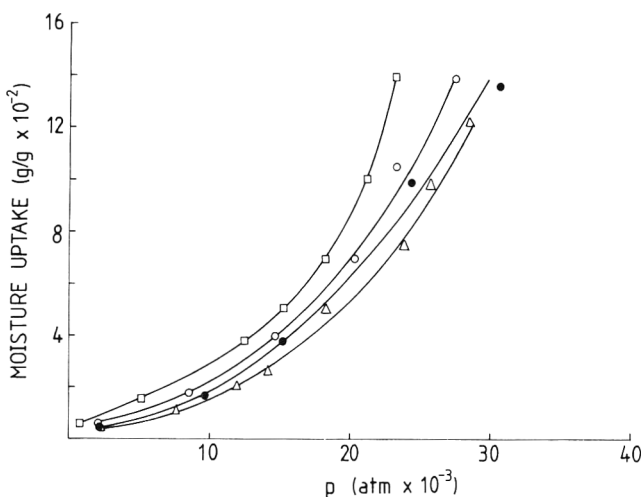

Fig. 8—Effect of crystalline sucrose addition on the sorption isotherm of cracker at $30°C$: □, cracker:sucrose (100:0); ○, cracker:sucrose (80:20); •, cracker:sucrose (50:50); △, sweet biscuit.

Fig. 9—Effect of crystalline sucrose addition on ΔH_s values in cracker system. ○, cracker:sucrsoe (100:0); □, cracker:sucrose (80:20): △, cracker:sucrose (50:50).

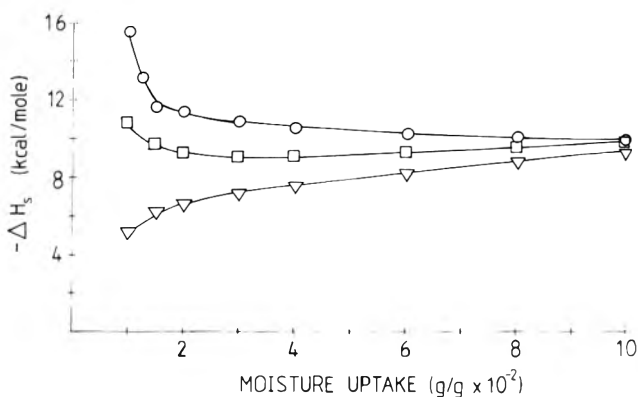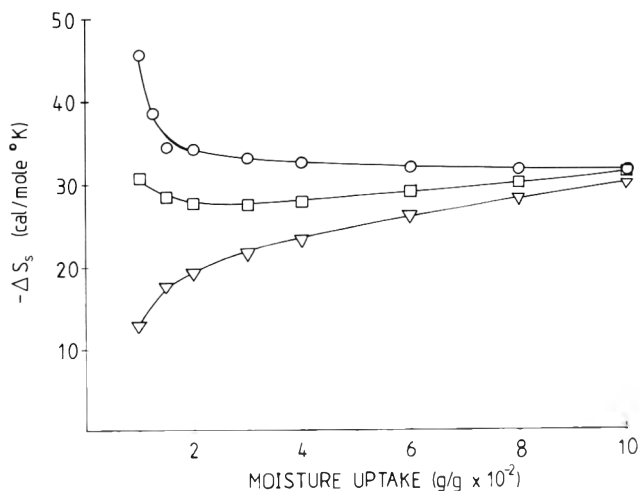Fig. 10—Effect of crystalline sucrose addition on ΔS_s values in cracker system at $30°C$. ○, cracker:sucrose (100:0); □, cracker:sucrose (80:20); △, cracker:sucrose (50:50).

Table 1—Free energy of sorption in cracker:crystalline sucrose and sweet biscuit systems at different moisture levels at 30°C

Moisture content (% d.b.)	ΔG$_s$ (kcal/mole)			
	Cr:Sucr. (100:0)	Cr:Sucr. (80:20)	Cr:Sucr. (50:50)	Sweet Bisc.
1	−1.57	−1.46	−1.23	−1.04
1.5	−1.15	−1.07	−0.94	−0.82
2	−0.97	−0.90	−0.79	−0.70
4	−0.67	−0.61	−0.54	−0.48
6	−0.55	−0.49	−0.43	−0.38
8	−0.47	−0.42	−0.37	−0.32
10	−0.42	−0.37	−0.32	−0.27

Table 2—Mean cluster size in cracker:crystalline sucrose and sweet biscuit systems at different moisture levels at 30°C

Moisture content (% d.b.)	Mean cluster size			
	Cr:Sucr. (100:0)	Cr:Sucr. (80:20)	Cr:Sucr. (50:50)	Sweet Bisc.
1	0.53	0.57	0.76	0.96
1.5	0.75	0.79	1.00	1.24
2	0.99	1.03	1.23	1.54
4	1.65	1.70	1.81	2.26
6	1.94	2.02	2.16	2.52
8	2.21	2.30	2.49	2.70
10	2.45	2.56	2.78	3.05

indicating a decreasing affinity for water. In the same way ΔH_s and ΔS_s values approached the respective values of the sweet biscuit system, (Fig. 9 vs Fig. 5, Fig. 10 vs Fig. 6). Fig. 9 and 10 clearly show how crystalline sucrose addition changed the shapes of the enthalpy and entropy functions of the cracker system. These data suggest that the water binding mechanism in the cracker system partly changed as a result of the crystalline sucrose additions; the less crystalline sucrose in the system the less the resulting active site binding giving typically high negative ΔH_s and ΔS_s values at low moisture contents.

Data on the men cluster sizes in the different cracker:crystalline sucrose systems are compared with the sweet biscuit system in Table 2. The data indicate that the more crystalline sucrose in the cracker system the greater the average cluster size at all moisture levels. This suggests that addition of crystalline sucrose made the cracker system less hydrophilic giving impetus to more water clustering.

Thus, isotherms, thermodynamic parameters, and the clustering analysis gave support to the validity of the hypotehsis that addition of crystalline sucrose in the cracker system changes the system's sorption characteristics closer to those of the sweet biscuit system.

REFERENCES

Apostolopoulos, D. and Gilbert, S.G. 1984. Water sorption of coffee solubles by inverse gas chromatography. Instrumental Analysis of Foods. Vol. 2.
Bettelheim, F.A., Elock, A., and Kaufman, L.J. 1970. Heats of water vapor sorption in swelling biopolymers. Biopolymers 9: 1531.
Bettelheim, F.A. and Volman, D.H. 1957. Pectic substances — water. 2. Thermodynamics of water sorption. J. Polym. Sci. 24: 445.
Chung, D.S. and Pfost, H.B. 1967. Adsorption and desorption of water vapor by cereal grains and their products. Trans. of the ASAE 10: 549.
Coelho, U., Miltz, J., and Gilbert, S.G. 1979. Water binding on collagen by inverse gas chromatography: thermodynamic considerations. Macromolecules 12: 284.
El-Warraky, A.G., Abdel-Rahman, N.R., El-Samkary, M.A., and Easa, A.A. 1980. Effect of sugar, fats and oils on biscuit quality. Research Bull. No. 1365. Faculty of Agriculture, Aim Shams Univ., Cairo, Egypt.
Everett, D.H. 1950. The thermodynamics of adsorption. Part 3. Analysis and discussion of experimental dta. Trans. Faraday Soc. 46: 957.
Flint, O. and Moss, R. 1970. A comparative study of the microstructure of different types of biscuits and their doughs. Food Trade Review 40(4): 32.
Francis, B. and Groves, C.H. 1962. A note on the application of histological techniques to the study of the structure of baked goods. J. Royal Microsc. Soc. 81: 53.
Gilbert, S.G. 1084. "Advances in Chromatography," Vol. 23, Ch. 6. Marcel Dekker, New York.

Gregg, S.J. and Sing, K.S.W. 1967. "Adsorption, Surface Area and Porosity." Academic Press, New York.
Helen, H.J. 1983. Moisture sorption of dry bakery products by inverse gas chromatography for package optimization. Ph.d. thesis. Rutgers Univ., Food Science Dept., New Brunswick, NJ.
Helen, H.J. and Aaltio, 1978. Uber die Wirkung von Verpackungsmaterialen auf die Haltbarkeit von Keksen: eine kritische Betrachtung und eine empirische Untersuchung. Forum Ware-Wissenschaft u. Praxis 6: 94.
Iglesias, H.A. and Chirife, J. 1976. Isosteric heats of water vapor sorption on dehydrated foods. Part 1. Analysis of the differential heat curves. Lebensm.-Wiss. u.-Technol. 9: 116.
Iglesias, H.A., Chirife, J., and Viollaz, P. 1976. Thermodynamics of water vapor sorption by sugar beet root. J. Food Technol. 11: 91.
Katz, E. and Labuza, T.P. 1981. Effect of water activity on the sensory crispness and mechanical deformation of snack food products. J. Food Sci. 46: 403.
Kiselev, A.V. and Yashin, Y.I. 1969. "Gas Adsorption Chromatography." Plenum Press, New York, NY.
Kissell, L.T., Marshall, B.D., and Yamazaki, W.T. 1973. Effect of variability in sugar granulation on the evaluation of flour cookie quality. Cereal Chem. 50: 255.
Kuntz, I.D. and Kauzman, W. 1974. Hydration of proteins and polypeptides. Adv. in Protein Chem. 28: 239.
Leiberman, E.R., Gilbert, s.G., and Srinivasa, V. 1972. The use of gas permeability as a molecular probe for the study of crosslinked collagen structures. Trans. N.Y. Acad. Sci., Ser. 11, Vol. 34: 694.
Nemitz, G. 1963. Die hygroskopischen Eigenschaften getrockneter Lebensmittel. Zeitschrift fur Lebensmittel-Untersuchung und -Forschung 123: 1.
Pomeranz, Y. 1973. "Wheat Chemistry and Technology." AACC Publ., St. Paul, MN.
Rockland, L. 1960. Saturated salt solutions for static control of relative humidity between 5° and 40°C. Anal. Chem. 32: 1375.
Rogers, C.E., Stannett, V.T., and Szwarc, M. 1959. The sorption of organic vapors by polyethylene. J. Phys. Chem. 63: 1406.
Starkweather, H.W. 1963. Clustering of water in polymers. Polymer Letters 1: 133.
Starkweather, H.W. 1975. Some aspects of water clusters in polymers. Macromolecules 8: 476.
Smith, D.S., Mannheim, C.H., and Gilbert, S.G. 1981. Water sorption isotherms of sucrose and glucose by inverse gas chromatography. J. Food Sci. 46: 1051.
Smith, D.S. 1982. The thermodynamic and structural aspects of water sorption by starches using inverse gas chromatography. Ph.D. thesis. Rutgers Univ., New Brunswick, NJ.
Young, D.M. and Crowell, A.D. 1962. "Physical Adsorption of Gases." Butterworts, Washington.
Zabik, M.E., Fierke, S.G., and Bristol, D.K. 1979. Humidity effects on textural characteristics of sugar-snap cookies. Cereal Chem. 56: 29.
Zimm, B.H. 1953. Simplified relation between thermodynamics and molecular distribution functions for a mixture. J. Chem. Phys. 21: 5.
Zimm, B.H. and Lundberg, J.L. 1956. Sorption of vapors by high polymers. J. Phys. Chem. 60: 425.
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Influence of Temperature and Time on Cooking Kinetics of Potatoes

T. HARADA, H. TIRTOHUSODO, and K. PAULUS

―――――――――― ABSTRACT ――――――――――

To study cooking kinetics, slices (6 mm thick, 30 mm diameter) of 3 potato varieties were treated in water at 90°, 100° and 110°C for varying times. Sensory, chemical and physical properties were investigated. Changes during cooking at constant temperature were mathematically described by a 0 order (texture, taste) and a 1st order (shear force, dry matter, pectin, soluble amylose, cell size) equation. The starch content remained constant. Temperature dependence of the rate constant could always be described by a first order equation. The z-values, which are necessary to calculate C-value for practical heat treatments, could be determined. Correlations established between kinetics of different properties showed that behavior of certain potato properties may be predicted by shear-force measurement.

INTRODUCTION

DESPITE SEVERAL ATTEMPTS to relate sensory data to objectively measured texture and/or chemical components in processed potatoes, there are still some unanswered questions. They concern both analytical problems and difficulties in interpreting the parameters measured. List and Emschermann (1981) reported that, in vegetables, the most important reaction during cooking was the change of insoluble protopectin into soluble hydropectin. Starch was found to be an essential component of potatoes. Kovacs et al. (1975) studied heat treatments of potatoes at temperatures up to 95°C. The rate of cooking, defined as the reciprocal of the cooking time required to reach a certain change in hardness was shown to be a linear function of the temperature. The Q_{10} values between 75° and 95°C were on average 2.0 in the majority of cases. Several authors have suggested the use of first order equations to describe softening of plant tissue during heating (Bourne, 1976; Sefa-Dedeh et al., 1978; Paulus and Saguy, 1980; Loh and Breene, 1981; Paulus and Tirtohusodo, 1982).

Experiments in this field frequently neglect the systematical aspects of experimentation; studies of cooking kinetics, for example, should include a wide range of cooking temperatuers and times. Therefore, in this research, the cooking behavior of potatoes was studied at 90°, 100° and 110°C in order to describe changes during cooking over the whole range of relevant temperature and time conditions.

MATERIALS & METHODS

Potato samples

The potato varieties Bintje, Mentor and Desiree, stored at 6 – 8°C up to 16 wk, were used in this study. Two days before the experiments, the potatoes were removed from storage for preparation of samples. For each treatment 100 slices (30 mm in diameter, 6.0 mm thick) were cut out of the core of 20 – 30 potato tubers and placed in wire baskets for cooking.

Thermal treatment

An apparatus especially designed for the experiments was used (Paulus et al., 1978) which allowed the slices to reach the required process temperature very rapidly and to cool rapidly after cooking. The slices were treated in approximately 16L water at the three temperatures – 90°, 100° and 110°C. Process times varied between 6 and 48 min at 90°C, 4 and 12 min at 100°C, and 1.5 and 4 min at 110°C. Cooking time was defined as the time elapsing from the moment when the product reached an average temperature of 80°C until the end of the heat treatment. The cooking time therefore is always shorter than the process time.

For an approximate calculation of the average temperature of the slices during processing the following equation was used:

$$\overline{\theta} = \frac{8}{\pi^2} \ e^{-(\frac{1}{2}\pi)^2 \frac{a \cdot t}{L^2}} \tag{1}$$

with

$$\theta = \frac{\tau_- - \overline{\tau}}{\tau_- - \tau_0} \tag{2}$$

where: τ_- = water temperature (90°, 100°, 110°C); τ_0 = product temperature (20°C) at t = 0; $\overline{\tau}$ = average product temperature; t = heating time; d = thickness of the slice; L = half thickness of the slice ($\frac{1}{2}$d = 3 · 10^{-3}m); a = thermal diffusivity (0.148 · 10^{-6} $m^2 s^{-1}$).

Different practical cooking processes take place at the varying temperatures. Hence the effect of each process must be described in a different way. The integral effect is calculated as follows: follows:

$$E = \int_0^t 10^{\frac{\tau - \tau_r}{z}} \cdot dt \tag{3}$$

where the integral effect E is the cook value C in this special case. The z-value characterizes the temperature dependence of the change in the property investigated, e.g. texture. τ is the product temperature and τ_r is the reference temperature. For cooking processes τ_r usually is 100°C.

Laboratory analyses

The treated slices were divided at random for chemical analysis, sensory evaluation and texture measurements.

Chemical analysis (40 slices). Dry matter content was determined gravimetrically after drying 10 – 15g of the mashed potatoes at 105°C for 4 hr.

Starch, pectin and soluble amylose contents as well as cell size were determined as reported elsewhere (Harada and Paulus, 1984).

Sensory evaluation (30 slices). A special category scale was used to assess taste and texture. The method is described in detail by Paulus and Tirtohusodo (1982) and Harada and Paulus (1984). Texture and taste of the cooked slices were analyzed by a trained panel of eight persons. The category scales for taste and texture comprised 11 categories (1 = completely raw/very hard, 6 = optimal taste/optimal texture, 11 = completely overcooked/pulpy). Each panel member recorded his numerical judgement of 3 slices per sample. White bread and light tea were available to panelists to rinse residual particulate matter from the mouth. The judgements for each given sample was averaged and used for regression analysis. The numerical judgement 6 corresponds to an optimally cooked sample. As texture is most important for evaluating the cooking degree, the time required to reach this value of texture at constant treatment temperature was called "optimal cooking time."

Texture measurement (30 slices). A universal testing machine (Zwick, model 1442) was used for shear-force measurements (Hara-

da and Paulus, 1984). Maximum shear force was used to characterize the effects of thermal treatment.

RESULTS & DISCUSSION

Texture and taste

The changes in texture and taste during cooking are shown in Fig. 1 and 2 for Mentor variety. These changes can be described by a 0 order relationship as follows:

$$S = a + b \cdot t \qquad (4)$$

where: S = numerical value of taste and texture judgement according to the category scale; t = process time (min); a = numerical value of taste and texture judgement at t = 0 being 1.0 theoretically; b = rate constant (min^{-1}).

The values for the coefficients a and b are compiled in Tables 1 and 2. Bintje variety required the shortest, and Desireé variety the longest cooking times at each of the three temperatures. The rate constants were similar for each sensory property in each variety, indicating that texture and taste, though independent properties, changed at similar rates at constant process temperatures. This means, if texture received optimal ratings, taste also reached a nearly optimal cooking degree. By using the optimal cooking times based on texture (Table 1) to calculate the corresponding numerical values of taste, one obtains an average value for taste of 6.3 ± 0.33.
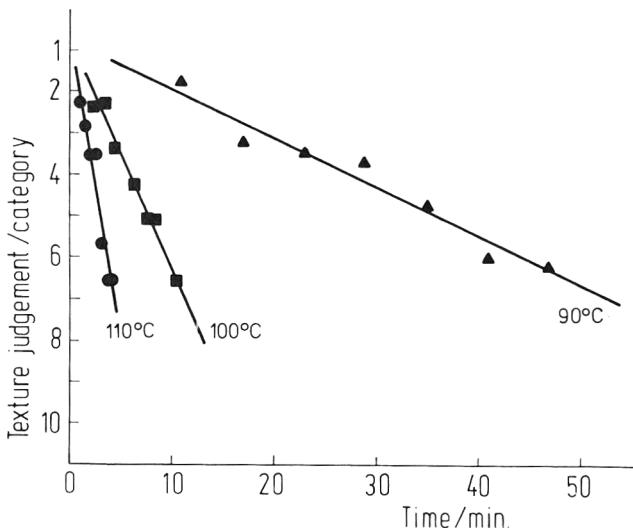
The temperature dependence of the changes in texture and taste can be expressed as follows:

$$\log b = -c + d \cdot \tau \qquad (5)$$

where: b = rate constant for texture and taste changes at constant temperature $(°C^{-1})$; τ = process temperature $(°C)$; c, d = coefficients.

The values for the coefficients are listed in Table 3. Furthermore, the z-values are tabulated; they are the reciprocal of the coefficient d in Eq. (5). These z-values are necessary to calculate the cook value, C, of a practical thermal process corresponding to Eq. (3). The z-value indicates how sensitively a component or property responds to temperature. Low values of z, or high values of d (Eq. 5) indicate that the temperature is very important. The influence of temperature on the rate constant, b, (Eq. 5) is also considerable, thus the intensity of a reaction or change is determined mainly by temperature. On the other hand high values of z or low values of d indicate that temperature is of secondary importance. The z-values for texture and taste are in an intermediary range and very similar. For microorganisms, for instance, z-values are much lower, for heat-resistant enzymes and most vitamins z-values are higher.

Shear force

Changes in shear force during cooking are shown in Fig. 3 for the variety Bintje. These changes can be described

Fig. 1—Development of texture during cooking of "Mentor" potatoes (1 = very hard; 6 = optimal texture; 11 = pulpy).
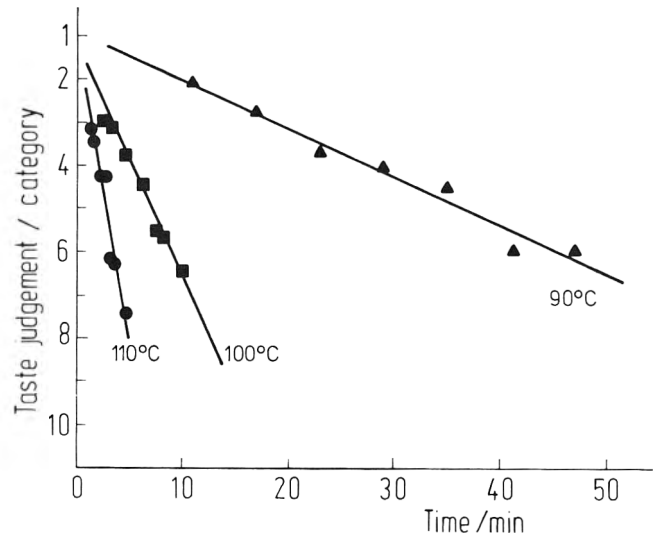
Fig. 2—Development of taste during cooking of "Mentor" potatoes (1 = completely raw; 6 = optimal taste; 11 = completely overcooked).

Table 1—Development of sensory texture during cooking (S = a + b · t)[a]

Variety	Temp (°C)	a —	b min^{-1}	n	r^2	optimal cooking time (min)
Bintje	90	1.09	0.141	9	0.90	34.8
	100	0.609	0.816	8	0.98	6.6
	110	0.562	1.52	8	0.88	3.6
Mentor	90	0.843	0.115	8	0.96	44.8
	100	0.761	0.548	9	0.98	9.6
	110	0.468	1.50	9	0.94	3.7
Desiree	90	1.13	0.097	6	0.94	50.2
	100	1.20	0.419	9	0.92	11.5
	110	0.779	1.32	8	0.90	4.0

[a] S = numerical value of texture; t = process time (min); a = texture value at t = 0; b = rate constant (min^{-1}); n = number of experiments (cooking times); r = correlation coefficient.

Table 2—Development of taste during cooking (S = a + b · t)[a]

Variety	Temp °C	a —	b min^{-1}	n	r^2
Bintje	90	1.50	0.144	8	0.98
	100	0.997	0.749	8	0.96
	110	0.724	1.49	9	0.95
Mentor	90	0.943	0.111	8	0.98
	100	1.10	0.542	9	0.96
	110	0.918	1.45	9	0.96
Desiree	90	1.42	0.099	7	0.88
	100	1.53	1.414	9	0.86
	110	1.01	1.42	9	0.88

[a] S = numberical value of taste; t = process time (min); a = taste value at t = 0; b = rate constant (min^{-1}); n = number of experiments (cooking times); r = correlation coefficient

mathematically by a first order equation, but a second order mechanism would also fit the measured shear-force data. In comparing the first order to the second order correlation, the average regression coefficients for the two equations are very similar for the same set of data. Using a first order equation has the advantage that the shear force of the raw material can be taken into account. This equation reflects the actual sitaution primarily in shorter process times. For process times exceeding the optimal cooking time, greater differences between calculated and real value exist, due to the fact that even overcooked samples still have a certain shear force This section of the shear-force development curve is preferably described by a second order equation. In practice, however, the process up to the optimal cooking time is of utmost importance, and therefore it seems justifiable to describe changes in shear force F with time t as follows:

$$\log F = a - b \cdot t \tag{6}$$

where: F = measured shear force (N); t = process time (min); a = shear force of the raw product (N): b = rate constant (min^{-1}).

The most important data are summarized in Table 4. The shear force at optimal cooking times was between 2 and 5N. This indicates a very narrow range as compared to untreated samples showing shear forces between 75 and 80N.

It is impossible, on the other hand, to determine from data obtained in only 3 varieties whether a certain shear force value indicates an optimal cooking effect for any potato variety. This question will be dealt with in future studies.

The z-values for shear force are listed in Table 3. It can be seen that the z-values for shear force are only slightly higher than those for sensory texture. It is therefore possible to evaluate the cooking effect on the basis of shear-force measurements if the relationship between shear force and sensory texture is known. This relationship may be expressed mathematically by combining Eq. (4) and (6) for each variety at each temperature. Averaging the data results in the following expression:

$$\log F = 1.99 - 0.26 \cdot S \tag{7}$$

The average shear force at optimal cooking is 2.7N.

Cooking effect

Despite similar z-values, different thermal treatments must be carried out to obtain the desired cooking effect in each of the three varieties.

Chemical components and cell size

The average data for the change from the raw into the optimally cooked state are summarized in Table 5. Dry matter and pectin contents were reduced, starch contents remained constant, cell size was increased, and soluble amylose contents were reduced during the thermal treatments. Possible explanations for these changes are given below.

All these changes were taking place as a function of temperature and time of the treatment and can be described mathematically. To express the influence of process time

Table 3—Temperature dependence of the alteration of shear force, sensory texture and taste (log b = − c + d • τ)[a]

	Variety	c	d $^\circ$C^{-1}	r^2	z-value $^\circ$C
Shear force	Bintje	5.86	0.049	0.96	20.4
	Mentor	6.36	0.054	0.94	18.5
	Desiree	6.24	0.052	0.96	19.2
Sensory texture	Bintje	5.42	0.052	0.92	19.2
	Mentor	5.92	0.056	0.96	17.9
	Desiree	6.10	0.057	1.0	17.6
Sensory taste	Bintje	5.34	0.051	0.94	19.6
	Mentor	5.93	0.056	0.96	17.9
	Desiree	6.20	0.058	1.0	17.2

[a] b = rate constant ($^\circ$C^{-1}); τ = process temperature ($^\circ$C); c, d = coefficients; r = correlation coefficient

Table 4—Mathematical description of shear force versus time during cooking (log F = a − b • t)[a]

Variety	Temp $^\circ$C	a N	b N • min^{-1}	n	r^2	F at t$_{opt}$ N
Bintje	90	1.71	0.039	7	0.91	2.1
	100	1.84	0.172	7	0.98	5.0
	110	1.89	0.389	8	0.96	3.1	3.4
Mentor	90	1.47	0.028	8	0.79	1.8
	100	1.80	0.168	7	0.95	1.6
	110	1.86	0.341	7	0.99	3.9	2.4
Desiree	90	1.70	0.029	7	0.92	1.8
	100	1.75	0.121	6	0.96	2.3
	110	1.88	0.327	7	0.94	3.4	2.4

[a] F = shear force (N); t = process time (min); t$_{opt}$ = optimal cooking time (min); a = log shear force of the raw product (N); b = rate constant (min^{-1}); n = number of experiments (cooking times); r = correlation coefficient.
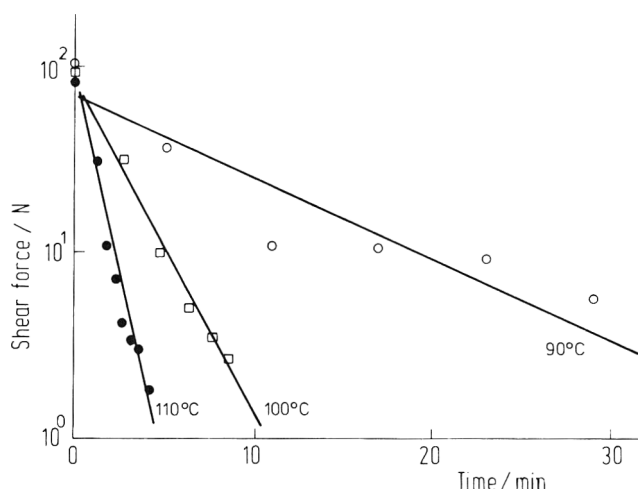

Fig. 3—Development of shear force during cooking of "Bintje" potatoes.

Table 5—Average values (all 3 varieties) of some constituents and properties for raw and optimally cooked potatoes

Constituent/ property		Raw potato slices	Optimally cooked potato slices
Texture judgement (−)		1.0 [a]	6.0 [a]
Shear force (N)		77	2.7
Dry matter content	(%)	19.8	17.4 ± 1.04
Starch content	(%)	13.9	13.9 ± 0.29
Soluble amylose content	(%)	—	0.118 ± 0.027
Pectin content	(%)	0.270	0.201 ± 0.016
Cell size	(µm)	143	212 ± 14.6

[a] These values were not determined, they were assessed by definition.

Table 6—Constants of Eq. (8) and data for the relative changes of the components and properties at optimal cooking[a]

Variety	Temp °C	Dry matter a %	10³b %·min⁻¹	r² —	$\frac{y_{opt}}{y_o}\cdot100$ %	Pectin a %	10²b %·min⁻¹	r² —	$\frac{y_{opt}}{y_o}\cdot100$ %	Soluble amylose a %	10³b %·min⁻¹	r² —	v_opt %	Cell size a μm	10²b μm·min⁻¹	r² —	$\frac{y_{opt}}{y_o}\cdot100$ %
Bintje	90	1.29	1.40	0.86	89	0.58	0.477	0.96	67	0.55	0.487	0.94	0.131	2.32	0.404	0.83	146
	100	1.30	5.80	0.86	92	0.56	1.59	0.94	78	0.55	1.83	0.85	0.108	2.35	2.03	0.90	138
	110	1.30	8.96	0.92	93	0.57	3.73	0.90	73	0.55	6.28	0.92	0.174	2.35	6.03	0.79	159
Mentor	90	1.20	1.31	0.83	88	0.53	0.362	0.86	70	0.54	0.471	0.88	0.145	2.34	0.486	0.88	159
	100	1.30	6.51	0.90	87	0.53	1.47	0.96	74	0.54	1.13	0.83	0.093	2.35	1.10	0.92	132
	110	1.30	9,67	0.77	92	0.53	3.11	0.92	78	0.54	2.84	0.96	0.075	2.33	5.26	0.76	158
Desiree	90	1.29	2.19	0.94	77	0.61	0.322	0.86	68	0.53	0.283	0.98	0.108	2.36	0.310	0.92	148
	100	1.29	6.84	0.98	84	0.61	0.875	0.90	78	0.53	1.28	0.94	0.112	2.36	1.11	0.92	140
	110	1.29	12.40	0.96	90	0.61	1.85	0.86	84	0.53	4.11	0.88	0.116	2.35	4.61	0.90	156

[a] y_{opt} = content at optimal cooking time; y_o = content of the unprocessed product.

Table 7—z-values for dry matter, pectin, soluble amylose content and cell size[a]

Variety	Dry matter z °C	r² —	Pectin z °C	r² —	Soluble amylose z °C	r² —	Cell size z °C	r² —
Bintje	25.0	0.93	21.8	0.99	18.1	1.00	17.2	0.99
Mentor	23.0	0.89	21.4	0.97	25.7	1.00	19.6	0.97
Desiree	27.0	0.97	27.0	0.99	17.2	0.99	17.2	1.00
Average	25.0	—	23.1	—	20.3	—	18.0	—

[a] r = correlation coefficient.

at constant process temperatures, first-order equations can be used of the general form:

$$\log y = a - b \cdot t \qquad (8a)$$
$$\log (y_{total} - y) = a - b \cdot t \qquad (8b)$$
$$\log (y_{max} - y) = a - b \cdot t \qquad (8c)$$

where: y = content of the constituent under consideration at time t; t = process time (min); a = content of the constituent under consideration in the unprocessed product; b = rate constant (min⁻¹).

In the case of dry matter and pectin, the absolute contents, y, are used (Eq. 8a). It seemed to be more reasonable in the case of soluble amylose to describe the difference between total amylose content, y_{total} (multiplying starch content by 0.25), and soluble amylose content, y, as a function of process time (Eq. 8b). Finally, in the case of cell size the difference between a maximum cell size, y_{max}, and the real cell size was used (Eq. 8c). The maximum cell size was found to be 365 μm for all three varieties.

The decrease in dry matter and pectin content, the reduction in soluble amylose content and the increase in cell size were calculated for all combinations of temperature and time with Eq. (8) and the corresponding constants are listed in Table 6. The last columns of Table 6 are most interesting, as these data apparently show a tendency towards similar relative changes during the treatment up to optimal cooking time. A comparison of these relative values for the components and properties at the optimal cooking point resulted in nearly identical values. They were averaged not only for one variety and different thermal treatments, but for all three varieties and treatments. Dry matter contents were reduced to 88.0 ± 3.7%, pectin contents to 74.4 ± 5.3%, cell size increased to 148 ± 7%. Soluble amylose was leached out up to about 3% of the original total amylose content.

The temperature dependence of the rate constants, b, could be again expressed corresponding to Eq. (5). For the coefficient, d, the z-values listed in Table 7 were calculated

as explained earlier. The components and properties of the three varieties investigated did not show the same behavior. The z-value of 18°C for cell size increase was the lowest, suggesting that cell size increase was the most temperature-sensitive property of all the properties investigated. Furthermore, this z-value is very close to those found for texture and taste (see Table 3).

CONCLUSIONS

A LABORATORY STUDY designed to assess cooking kinetics for three potato varieties was conducted. For all constituents (dry matter, starch, soluble amylose, pectin) and properties (cell size, texture, taste, shear force) a mathematical description of the changes during thermal treatment was possible.

Changes in the most important sensory properties, texture and taste, with time at constant process temperature could be expressed by a zero order equation. The kinetics, including temperature dependence of the changes, are very similar. As texture is the most important sensory property to show cooking effects, and, as kinetics of taste changes are very similar, the sensory texture judgements were used to define the different cooking degrees and to asses an optimal cooking time.

The changes in shear force with treatment time at constant process temperature followed a first order relationship. The temperature dependence of this mechanism is similar to that for texture. Therefore, a good correlation was found to exist between shear force and sensory texture. Thus, changes in texture could be described and controlled by a corresponding objective shear force measurement. The shear forces of raw potatoes differed somewhat from variety to variety and also the shear forces at optimal cooking time were somewhat different.

For dry matter, soluble amylose, pectin content and cell size, the changes at constant temperatures could also be characterized by first order equations. Starch contents were not influenced during processing. The temperature dependence had varying influence on physical properties, cell-size increase being most dependent, and reduction in dry matter content being least dependent upon temperature. For the individual parameters investigated, the same relative values were calculated at optimal cooking time for all three varieties. The temperature-time integral over the whole process, according to the definition of the cooking degree, is the decisive measure for the effects of a thermal treatment.

The data obtained do not allow, however, the general prediction of cooking behavior of any variety from its chemical composition. The coefficients and constants in the mathematical expressions applied must be determined for each individual case.

Influence of the Composition of Potatoes on Their Cooking Kinetics

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———————————ABSTRACT———————————

A previous study of three potato varieties indicated that shear force measurement may be used to predict the behavior of certain potato properties during cooking. To verify this hypothesis and to confirm other previous findings, slices (6 mm thick, 30 mm in diameter) of 21 potato varieties were thermally treated in water at 100°C. Mathematical expressions were assessed, and coefficients were determined to describe the kinetic behavior of the varieties.

INTRODUCTION

FOR PRACTICAL PURPOSES, particularly for the potato-processing industry or catering institutions, it is important to develop simple methods allowing prediction of the behavior of potatoes during defined thermal treatments.

Results obtained with 3 varieties — Bintje, Mentor and Desiree — have shown that changes in properties taken into account (sensory properties, chemical and physical properties), may be described by similar mathematical relationships (Harada et al., 1985). The sensory properties texture and taste, and also the objective criterion of shear force, showed nearly the same temperature dependence of the pertinent rate constant. A correlation between shear force and sensory texture would offer a possibility for replacing sensory tests since simple and rapid texture measurements would provide sufficiently precise information on the cooking behavior of a certain potato variety.

In order to verify such a hypothesis, the correlation between subjective and objective texture properties should first be studied in a broad spectrum of potato varieties. Furthermore it seemed of interest to look for further correlations between the properties investigated, including composition of the raw material, in order to obtain explanations for the cooking behavior and cooking kinetics of potatoes.

MATERIALS & METHODS

Potato samples

Twenty-one potato varieties obtained from both the domestic and imported markets were used. Tubers purchased from wholesale traders had not been stored previously. One hundred slices (6 mm thick, 30 mm in diameter) from 20 – 30 potato tubers were used for one treatment and subsequent investigations.

Thermal treatment

Potato slices were treated in water at 100°C. Process times varied from 1 min to 30 min and included the range of distinct overcooking. Equipment and processing techniques were reported by Paulus et al. (1978) and Harada et al. (1985).

Chemical, physical and sensory analyses

The following properties were determined in all of the 21 varieties: specific weight, cell diameter, dry matter, starch, pectin, shear

force, texture and taste. The methods used were discussed in detail elsewhere (Harada and Paulus, 1985; Harada et al., 1985).

Previous experiments had shown that dry matter and pectin contents changed considerably during cooking, whereas starch content was not influenced by the treatment. The starch content therefore was not investigated in the treated samples, but was used to characterize the raw material.

RESULTS & DISCUSSION

Raw potatoes

Data characterizing the composition of potatoes and cell sizes are compiled in Table 1. There were considerable differences among the 21 varieties. Specific weights varied between 1.061 and 1.096 g/cm^3, and dry matter contents between 15.2 and 22.5%. Starch contents were between 10.1 and 17.4%. Pectin levels showed variety-specific fluctuations ranging from 0.170 – 0.290%. Cell diameters varied from 132 – 171 μm. In view of this great variability in the raw material, the spectrum of varieties used could be regarded as representative of potatoes available in the market.

Shear forces of raw tuber slices were between 56.3 and 83.4N. These values confirm considerable differences in the firmness of the raw material.

Texture and taste were not determined in raw slices. The category scale for sensory analyses of these two properties comprised 11 categories, from 1 = completely raw/very

Table 1—Chemical analysis of raw potato slices

Variety	Specific gravity g/cm^3	Dry matter %	Starch %	Pectin %	Cell size μm
Bintje	1.088	19.8	14.1	0.273	145
Mentor	1.087	20.0	13.9	0.290	145
Desiree	1.081	19.5	13.6	0.248	139
Saskia	1.070	15.9	10.3	0.257	133
Nicola	1.073	17.9	13.2	0.212	140
Spunta	1.076	18.1	14.5	0.202	132
Jearla	1.076	17.0	13.8	0.215	146
Christa	1.069	15.5	10.8	0.206	147
Atica	1.065	15.2	10.8	0.221	166
Sieglinde	1.096	22.3	16.6	0.200	151
Ulla	1.079	21.2	16.3	0.234	144
Wilja	1.074	19.9	15.6	0.190	139
Clivia	1.070	19.1	13.2	0.248	164
Selma	1.078	19.8	14.1	0.215	138
Grata	1.082	20.0	15.1	0.170	145
Culpa	1.080	19.3	14.4	0.207	156
Grandifolia	1.092	20.0	15.0	0.200	150
Aula	1.094	22.5	17.4	0.238	169
Granola	1.081	20.9	15.7	0.197	158
Irmgard	1.085	18.5	14.2	0.210	171
Isola	1.061	15.6	10.1	0.217	164
$\bar{x}$	1.078± 0.009	19.0± 2.1	13.9± 2.0	0.221± 0.028	150±12
Max.	1.096	22.5	17.4	0.290	171
Min	1.061	15.2	10.1	0.170	132

hard to 11 = completely overcooked/pulpy; 6 = optimally developed property. According to these categories, the sensory quality of the raw slices was fixed at 1 for texture and taste.

Average values of properties and constituents evaluated are listed in Table 2.

Processed potatoes

Dry matter, pectin, cell diameter. Data obtained for 6 - 10 process times of different lengths at 100°C for each variety corresponded to a first order equation describing these properties as a function of process time.

Changes in dry matter content, which decreased during the treatment, are compiled in Table 3. The first two columns show the constants for the respective equation, whereas the last two columns indicate the corresponding data for optimal cooking times. The variability of the rate constant b is of primary interest as this constant provides a value for the intensity of changes. A pronounced difference in the rate of dry matter decrease was evident. A comparison of varieties Nicola and Culpa revealed that the rate of substance losses differed by as much as a factor of 3. As the correlation coefficients confirmed, however, these changes may be adequately described by the mechanisms selected.

Table 2—Average values (all varieties) of some constituents and properties for raw and optimally cooked potatoes

Constituent/property		Raw potato slices	Optimally cooked potato slices
Texture judgment	(—)	1.0[a]	6.0[a]
Shear force	(N)	71.4 ± 7.9	2.73 ± 1.4
Dry matter content	(%)	19.0 ± 2.1	16.7 ± 1.9
Pectin content	(%)	0.221 ± 0.028	0.172 ± 0.023
Cell size	(μm)	150 ± 12	203 ± 11.3

[a] These values were not determined, they were assessed by definition.

Table 4—Mathematical description of the changes in pectin content (%) during cooking (log y = a − b × 10^{-2} t)[a]

Variety	a %	b % min⁻¹	n	−r	y at t_{opt} %	$\dfrac{y_{t_{opt}}}{y_{t=0}} \cdot 100$ %
Bintje	0.564	1.59	8	0.97	0.214	78.4
Mentor	0.526	1.37	9	0.98	0.214	73.8
Desiree	0.612	0.875	8	0.95	0.193	77.8
Saskia	0.584	0.977	10	0.95	0.208	80.9
Nicola	0.686	0.860	9	0.92	0.162	76.4
Spunta	0.699	1.09	9	0.93	0.150	74.3
Jearla	0.660	1.65	7	0.90	0.169	78.6
Christa	0.691	0.926	9	0.92	0.167	81.1
Atica	0.662	2.08	6	0.96	0.175	79.2
Sieglinde	0.700	1.15	6	0.97	0.156	78.0
Ulla	0.634	1.37	6	0.95	0.178	76.1
Wilja	0.723	1.18	7	0.99	0.147	77.4
Selma	0.671	1.29	8	0.92	0.167	77.7
Grata	0.779	1.52	8	0.94	0.130	76.5
Culpa	0.688	1.55	7	0.96	0.155	74.9
Grandifolia	0.705	1.54	7	0.90	0.156	78.0
Aula	0.625	1.70	6	0.97	0.194	81.5
Irmgard	0.673	1.87	6	0.98	0.169	80.5
Isola	0.664	1.68	7	0.98	0.164	75.6
	— 1.39 ± 0.340	—		— 0.172 ± 0.023	77.7 ± 2.2

[a] y = pectin content (%); a = log of the theoretical pectin content at t = 0; t = process time (min); t_{opt} = optimal process time (min); b = rate constant (% min⁻¹); n = number of experiments; r = correlation coefficient.

The same general mathematical relationships could be applied to describe changes in pectin content (Table 4) and cell size (Table 5). The last columns of these tables, showing the relative changes at optimal cooking time compared to nonprocessed samples, were of special interest. It became obvious from these dates that the relative changes during thermal treatment were very similar in all

Table 3—Mathematical description of the changes in dry matter content (%) during cooking (log y = a − b × 10^{-3}t)[a]

Variety	a %	b % min⁻¹	n	−r	y at t_{opt} %	$\dfrac{y_{t_{opt}}}{y_{t=0}} \cdot 100$ %
Bintje	1.302	5.80	7	0.93	18.2	91.9
Mentor	1.300	6.51	8	0.96	17.1	85.5
Desiree	1.293	6.84	9	0.99	16.2	83.1
Saskia	1.204	5.25	10	0.87	14.2	89.3
Nicola	1.242	3.77	9	0.83	15.6	87.2
Spunta	1.250	4.81	9	0.87	15.6	86.2
Jearla	1.234	5.98	7	0.91	15.4	90.6
Christa	1.187	5.96	8	0.92	13.6	87.7
Atica	1.184	6.02	6	0.90	14.4	94.7
Sieglinde	1.346	5.17	6	0.98	20.2	90.6
Ulla	1.323	4.91	6	0.96	19.0	89.6
Wilja	1.301	7.25	8	0.98	17.2	86.4
Selma	1.296	5.17	8	0.91	18.2	91.9
Grata	1.309	7.32	8	0.97	17.8	89.0
Culpa	1.284	11.7	7	0.96	15.4	79.8
Grandifolia	1.302	8.14	7	0.95	17.6	88.0
Aula	1.352	7.47	6	0.97	20.4	80.7
Irmgard	1.266	4.74	7	0.93	17.5	94.6
Isola	1.193	4.30	7	0.96	14.5	92.9
	— 6.16 ± 1.73	—		— 16.7 ± 1.9	88.9 ± 3.7

[a] y = dry matter content (%); a = log of the theoretical dry matter content at t = 0; t = process time (min); t_{opt} = optimal process time (min); b = rate constant (% min⁻¹); n = number of experiments (cooking times); r = correlation coefficient.

Table 5—Mathematical description of the changes in cell size (μm) during cooking (log (y_{max} − y) = a − b × 10^{-2} t)[a]

Variety	a μm	b μm min⁻¹	n	−r	y at t_{opt} μm	$\dfrac{y_{t_{opt}}}{y_{t=0}} \cdot 100$ %
Bintje	2.352	2.03	8	0.95	199	137
Mentor	2.346	1.10	8	0.96	191	132
Desiree	2.359	1.11	8	0.96	194	140
Saskia	2.375	1.94	8	0.96	211	159
Nicola	2.349	1.13	9	0.90	199	142
Spunta	2.363	2.21	8	0.97	235	178
Jearla	2.336	2.06	6	0.98	210	144
Christa	2.337	1.66	9	0.89	216	147
Atica	2.295	1.88	6	0.90	204	123
Sieglinde	2.326	1.42	5	0.99	206	136
Ulla	2.343	1.12	6	0.99	186	129
Wilja	2.353	1.84	6	0.99	211	152
Selma	2.357	1.31	6	0.98	187	136
Grata	2.341	1.49	7	0.94	1.94	134
Culpa	2.322	1.37	7	0.90	201	129
Grandifolia	2.328	1.75	6	0.97	205	137
Aula	2.288	2.06	6	0.99	213	126
Irmgard	2.290	1.61	6	0.94	204	119
Isola	2.306	1.11	6	0.98	196	120
	— 1.59 ± 0.371	—		— 203 ± 11.3	138 ± 13.8

[a] y = cell size (μm); y_{max} = maximal cell size (μm); a = log of the theoretical cell size at t = 0; t = process time (min); t_{opt} = optimal process time (min); b = rate constant (μm · min⁻¹); n = number of experiments; r = correlation coefficient.

varieties tested; the corresponding values at optimal cooking times could therefore be averaged. The low standard deviations of these mean values indicated that there seemed to be no varietal influence as far as changes in these constituents and properties were concerned. The apparent contradiction to the quite different values for the rate constant, b, could be explained by different times required to reach the optimal cooking point.

Texture and taste. The results of the calculated changes in the most important sensory properties texture and taste, based on the experimental data, are summarized in Tables 6 and 7. For both properties the changes at a constant process temperature of $100°C$ could be described as a linear function of time. The high correlation coefficients confirm that both sensory method and mathematical treatment of the results were sufficiently precise to characterize the development of taste and texture during cooking.

Previous studies with three other varieties had revealed that the rate constants for texture and taste development at constant process temperatures for the same variety were relatively similar; the kinetics of changes in taste and texture, hence, corresponded well (Harada et al., 1985).

This funding can be generalized as the values for rate constant b for taste and texture development in Tables 6 and 7 are very similar within the same variety.

Optimal cooking time was defined as the process time necessary to obtain a judgement of sensory texture corresponding to category 6. These optimal cooking times for the 21 varieties which were calculated on the basis of the individual equations for texture are listed in the last columns of Table 6. Rate constants for texture changes varied considerably, and this is the reason for the extremely different optimal cooking times. Cooking times varied for the same process conditions from 4.5 min for variety Atica to nearly 12 min for varieties Nicola and Desirée.

The last column of Table 7 contains the calculated taste values for optimal process times. These values are very close to category 6 which has been described as the optimal taste category. This underlines the finding mentioned before that texture and taste showed very similar kinetics during cooking, both in a qualitative and quantitative sense.

Shear force. The decrease in shear force of potato slices with increasing time of thermal treatment could be described by first order or second order equations, as previous studies had shown. Advantages and disadvantages of the two mechanisms have been previously explained (Harada et al., 1985).

Table 8 contains the data for either approach. They confirm the results obtained in studies with only three varieties. Either approach obviously was suitable to keep the sum of the distance squares low, as all correlation coefficients were very close to 1.0. If the calculated shear force values for the optimal cooking times are compared, one finds a tendency towards lower values from use of a first order equation. The description by second order equation, corresponding to a flatter curve with longer cooking times, therefore, is more suitable to describe conditions of over-cooking. When the mean values of shear forces at optimal cooking times were compared to the actually measured shear forces at optimal cooking times, the following results were obtained: First order mechanism: $F = 2.55 \pm 1.3N$; Second order mechanism: $F = 3.66 \pm 1.2N$; Measured at t_{opt}: $F = 3.15 \pm 0.93N$.

This comparison shows that for the part of the thermal process up to the point of optimal cooking, which usually is of practical significance, the simpler mathematical description of a first order equation had no disadvantage, but offered the advantage of including also the initial values of nonprocessed potatoes. For times $t > t_{opt}$ use of a second order mechanism was justifiable as well, however. But independent of the kind of mathematical description used, shear forces for the optimal cooking state were close to each other. Mean value and standard deviation of the actually measured values were between 2 and 5N indicating a relatively narrow range.

Correlations

Texture and shear force. On the basis of either parameter being represented as a function of time, a first order equation was obtained as mathematical description, i.e. the logarithm of shear force, F, could be described as a function of texture evaluation, S. This is shown in Fig. 1. The equation for this curve, which was based on 163 value pairs, is:

Table 6—Development of sensory texture during cooking $(S = a + b \cdot t)$[a]

Variety	a —	b min^{-1}	n	r	Optimal cooking time min
Bintje	0.609	0.816	8	0.99	6.61
Mentor	0.761	0.548	9	0.99	9.56
Desiree	1.20	0.419	9	0.96	11.5
Saskia	1.13	0.495	10	0.95	9.84
Nicola	1.16	0.418	9	0.97	11.6
Spunta	1.03	0.440	8	0.97	11.3
Jearla	0.690	0.766	6	0.99	6.93
Christa	1.03	0.518	8	0.99	9.59
Atica	0.647	1.17	6	0.96	4.58
Sieglinde	1.23	0.531	5	0.99	8.98
Ulla	0.900	0.621	8	1.00	8.21
Wilja	1.05	0.543	9	0.99	9.12
Clivia	1.27	0.708	7	0.99	6.68
Selma	1.33	0.570	9	0.96	8.19
Grata	1.12	0.682	8	0.99	7.16
Culpa	1.41	0.597	8	0.98	7.69
Grandifolia	1.44	0.669	8	0.98	6.82
Aula	0.892	0.999	6	1.00	5.11
Granola	1.50	0.660	7	0.98	6.82
Irmgard	1.09	0.948	7	1.00	5.18
Isola	1.31	0.660	7	0.98	7.05

[a] S = numerical value of texture; t = process time (min); a = texture value at t = 0; b = rate constant (min^{-1}); n = number of experiments (cooking times); r = correlation coefficient.

Table 7—Development of taste during cooking $(S = a + b \cdot t)$[a]

Variety	a	b min^{-1}	n	r	S at t_{opt}
Bintje	0.997	0.749	8	0.98	6.0
Mentor	1.10	0.542	9	0.98	6.3
Deisree	1.53	0.414	9	0.93	6.3
Saskia	1.46	0.478	10	0.99	6.2
Nicola	1.56	0.440	9	0.94	6.6
Spunta	1.14	0.465	8	0.93	6.4
Jearla	1.11	0.685	6	0.98	5.9
Christa	1.27	0.570	8	0.95	6.7
Atica	0.781	1.10	6	0.96	5.8
Sieglinde	1.35	0.541	5	0.99	6.2
Ulla	1.02	0.631	8	1.00	6.1
Wilja	1.10	0.548	9	0.98	6.1
Clivia	1.60	0.639	7	0.96	5.9
Selma	1.50	0.568	9	0.95	6.1
Grata	1.09	0.729	8	0.98	6.3
Culpa	1.67	0.571	8	0.96	6.0
Grandifolia	1.57	0.674	8	0.97	6.2
Aula	0.934	1.01	6	0.99	6.1
Granola	1.56	0.676	8	0.96	6.2
Irmgard	1.10	0.904	6	1.00	5.8
Isola	1.23	0.714	7	1.00	6.3

[a] S = numerical value of taste; t = process time (min); a = taste value at t = 0; b = rate constant (min^{-1}); n = number of experiments (cooking time); r = correlation coefficient.

$$\log F = 1.919 - 0.246\,S \qquad r = -0.884 \qquad (1)$$

For the optimal texture value (S = 6) a shear force of 2.8N was obtained which was in good correspondence to the mean value of the actual shear foce at the point of optimal cooking (3.2N). From use of a second order equation, the shear force was 3.6N.

This correlation can be used to save expensive sensory analyses. Fig. 1 shows that it will scarcely be possible to predict the cooking behavior of an individual potato variety exactly on the basis of a single shear force measurement only. But an approximate assessment of the cooking behavior could be obtained from two shear force measurements as explained in the following. The two shear force values measured at two different treatment times allow constants a and b to be determined in the equation describing shear force F as a function of process time (see Table 8):

$$\log F = a + b \cdot t \qquad (2)$$

This equation (2), then, is the variety-specific relationship valid only for the potatoes under consideration. But the two measured shear force values can be used to determine the corresponding texture values through the relationship drawn in Fig. 1 which is valid for any potato variety. With the resulting values for texture, S, and the corresponding process times, the coefficients in the correlation between S and t (Table 6)

$$S = a + b \cdot t \qquad (3)$$

can be calculated. In this way the two mathematical descriptions for a certain variety of potatoes were defined, and the optimal cooking time could now be determined by the correlation between sensory texture, S, and treatment time, t, for which coefficients a and b are known, by using the value of 6.0 for S. It should be noted, however, that this is possible only if the same geometry of potato slices and the same test arrangement for texture measurements are applied.

Cell diameter of raw potatoes and cooking time. Of the parameters considered for predicting the cooking behavior from the raw material, mean cell diameter seemed to be a suitable criterion. The correlation is shown in Fig. 2. The equations correlating cell diameter C and optimal cooking time, t_{opt}, are

$$C = 186.76 - 4.63 \cdot t \qquad r = -0.804 \qquad (4)$$

$$t_{opt} = 28.93 - 0.140C \qquad r = -0.804 \qquad (5)$$

The graph and equations show that it is possible to predict optimal cooking time with qualifications. Again, the correlation is valid only for the geometry used in these experiments.

Beside the exact description of cooking kinetics, this relationship gave a highly interesting qualitative result. Obviously the cell size of raw potatoes is an important criterion for the behavior of potatoes during thermal treatments in water. The smaller the cell size the longer the time necessary to obtain an optimally cooked product, and the larger the cell size the shorter this time. As cell size is a variety-specific property, this relationship could be used for selecting potato varieties for thermal treatments to obtain a desired effect of the treatment.

Composition and cooking behavior

Many attempts were made to correlate the cooking behavior with certain components, or, more generally, to the composition of the product. Williams (1963) reported that cell wall ingredients decreased with increasing dry matter content. Keijbets (1974) reported decreasing pectin content and specific weight on the other. A correlation of ingredients to each other seems only partially suitable to predict the cooking behavior.

There are many reports and surveys dealing with the influence of individual ingredients on the breakdown of potatoes (Linehan and Hughes, 1969a, b; Warren et al., 1975; Warren and Woodman, 1974; Reeve, 1977; Hadziyev and Steele, 1979). The influence of cell size or texture was reported by Barrios et al. (1963) and by Linehan et al. (1968). Barrios assumed that large cells led to a distinct mealiness after cooking, and Linehan concluded that potatoes with larger cells tended more to breaking down during cooking.

Fig. 3 shows the correlation between shear force and dry matter content of potatoes at optimal cooking time. It

Table 8—Mathematical description of the change in shear force F during cooking[a]

Variety		$\log F = a - bt$						$\log F = a - b \cdot \log t$				Measured shear force at or near t_{opt}. N
	n	a N	b N/min	r	$F_{t=0}$ N	$F_{t=opt}$ N	n	a N	b N/min	r	$F_{t=opt}$ N	
Bintje	7	1.844	0.172	0.99	77.8	5.0	6	2.222	1.903	1.00	4.6	5.1
Mentor	7	1.804	0.168	0.97	76.6	1.6	6	2.136	1.808	0.99	2.3	3.3
Desiree	6	1.766	0.129	0.98	79.0	1.9	5	1.972	1.484	0.99	2.5	2.3
Saskia	11	1.682	0.171	0.95	76.2	1.0	10	1.549	1.284	0.99	1.9	2.1
Nicola	11	1.498	0.127	0.91	67.1	1.0	10	1.396	1.018	0.98	2.1	2.2
Spunta	9	1.575	0.174	0.17	56.3	0.4	8	1.338	0.991	0.98	2.0	2.5
Jearla	8	1.539	0.222	0.97	72.5	1.0	7	1.533	1.186	0.96	3.4	3.6
Christa	10	1.634	0.171	0.98	65.4	1.0	9	1.412	1.065	0.96	2.3	2.0
Atica	7	1.781	0.286	0.99	69.1	2.9	6	1.374	1.256	0.92	3.5	2.7
Sieglinde	11	1.841	0.150	1.00	73.0	3.1	10	1.714	1.083	0.95	4.8	3.6
Ulla	11	1.843	0.149	0.99	79.0	4.1	10	1.706	1.061	0.94	5.4	3.7
Wilja	12	1.771	0.144	0.97	77.2	2.8	11	1.674	1.145	0.95	3.8	3.3
Clivia	9	1.726	0.202	0.99	65.7	2.4	8	1.484	1.192	0.97	3.2	2.7
Selma	10	1.799	0.164	0.99	57.5	2.9	9	1.615	1.049	0.95	4.5	2.7
Grata	10	1.732	0.189	1.00	62.1	2.4	9	1.552	1.017	0.94	4.8	3.3
Culpa	10	1.787	0.171	1.00	73.4	3.0	9	1.588	1.087	0.94	4.2	3.1
Grandifolia	9	1.779	0.195	1.00	64.0	2.9	8	1.571	1.138	0.93	4.2	3.0
Aula	8	1.869	0.239	1.00	83.4	4.4	7	1.549	1.220	0.94	4.8	5.4
Irmgard	8	1.769	0.207	0.99	64.5	4.9	7	1.496	1.064	0.94	5.4	4.2
Isola	9	1.772	0.205	0.98	78.0	2.1	8	1.514	1.191	0.96	3.2	2.5
Granola	9	1.856	0.206	0.99	82.4	2.7	8	1.609	1.219	0.95	3.9	2.8

[a] F = shear force (N); t = process time (min); a = log shear force of the raw material (N); b = rate constant (N min^{-1}); r = correlation coefficient.
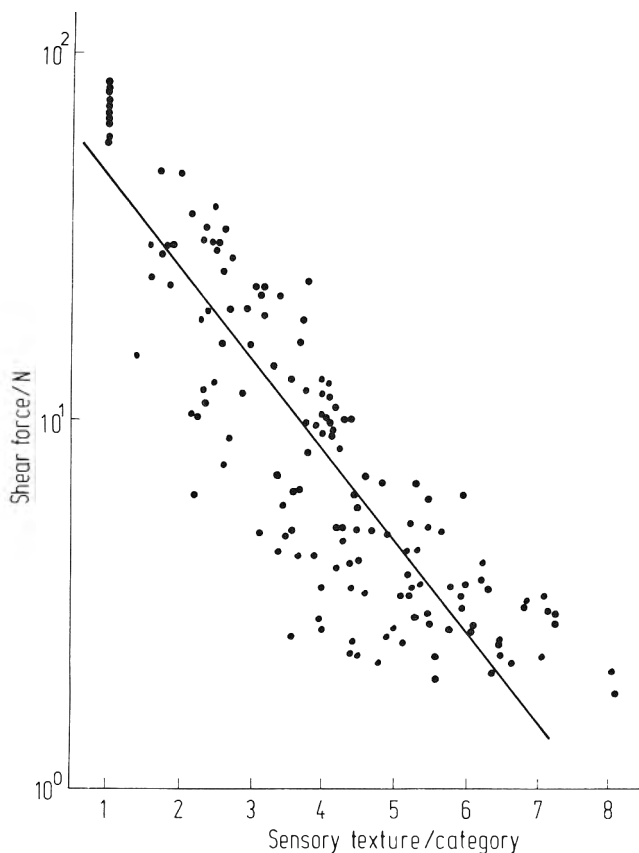
Fig. 1—Correlation between shear force and sensory texture (21 varieties, n = 163; category 1 = very hard; 6 = optimal texture, 11 = pulpy). $\log F = 1.919 - 0.246\,S$, $r = -0.88$.
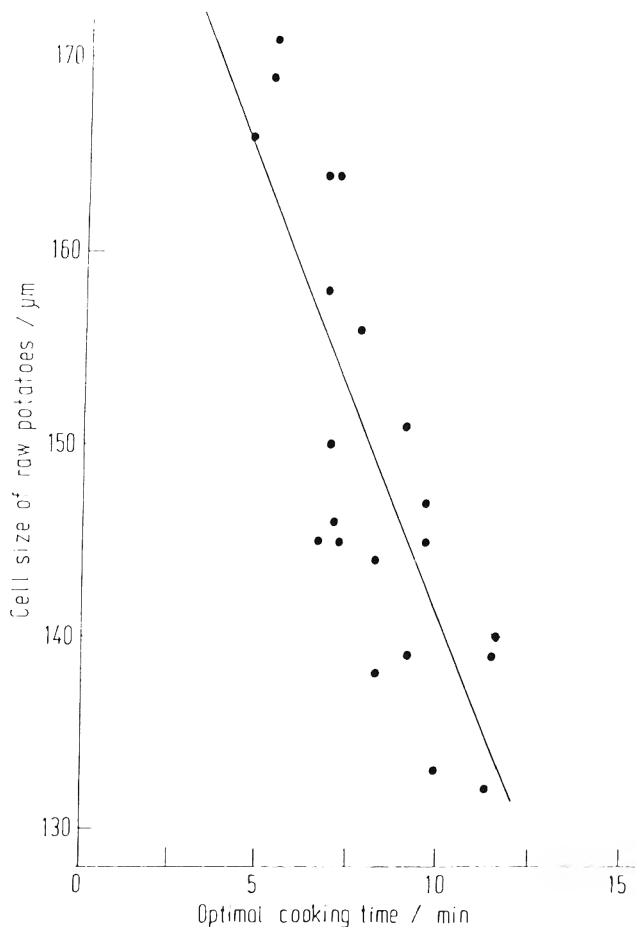

Fig. 2—Correlation between cell size of raw potatoes and optimal cooking time (21 varieties, n = 21). $C = 186.76 - 4.63\,t_{opt}$, $r = -0.80$; $t_{opt} = 28.93 - 0.140\,C$, $r = -0.80$.

should be noted that shear forces for the individual varieties, as mentioned before, were in a relatively narrow range of about 1 – 5N. A correlation to the corresponding dry matter content could be established, although the correlation coefficient was not very high.

There were no convincing correlations between pectin content and cooking behavior although pectin has been frequently mentioned as an important factor (Reeve, 1977).

Changes in cell diameter, however, seemed to be a suitable parameter for explaining the cooking behavior. In addition to the already discussed correlation between cell diameter and time necessary for optimal cooking (Fig. 1), Fig. 4 shows that the relative increase in cell diameter was paralleled by a relative decrease in dry matter content.

Each living cell has a certain potential for cell extension which is effective only, however, if the necessary space is available. A cell can increase in size and volume only if other cells provide this necessary space. Thermal treatments lead to mechanical damage, i.e., cells burst; the osmotic system is affected, if not destroyed; starch grains break or dissolve. Cells which have been damaged in this way decrease considerably in volume or are completely lost while others take their place. In this way also the stability of the entire system is reduced, as binding capacity and forces between individual cells decrease until finally the tissue disintegrates in the state of extreme overcooking.

CONCLUSIONS

CHANGES in dry matter content, pectin content, and cell diameter of potato slices during thermal treatment in water are described by a first order equation. The constants of

this equation differed from variety to variety. Substance losses per unit of time during cooking may differ by as much as a factor of 3. But, as varieties with a high value for the rate constant had a short cooking time, and vice versa, relative changes within the varieties up to the point of optimal cooking were similar.

Changes in the most important sensory properties, texture and taste, could be expressed by a zero order equation. Rate constants for texture and taste were very similar, and, therefore, texture may be used as an indicator property. Optimal cooking time, assessed on the basis of texture, varied from 4.5 – 11.5 min.

The description of the decrease in shear force during cooking was more complicated. Up to the optimal cooking time the data fitted a first order equation. For treatments beyond the optimal cooking time, second order equations were more suitable, as they more precisely describe the very flat curve for obtained longer cooking times. Average shear force decreased from 72N for raw slices to 3N for optimally cooked ones.

The correlation between texture and shear force was mathematically expressed. This equation could be used to check the cooking behavior of an unknown variety. With two shear-force measurements, the optimal cooking times could be approximated.

A very interesting finding was that a close relationship exists between cell size of the raw potatoes and optimal cooking time: the smaller the cell diameter, the longer the cooking times. Cell diameter, hence, offered a possibility for explaining the cooking behavior of potatoes. Cell diameters of the raw material allowed the necessary cooking
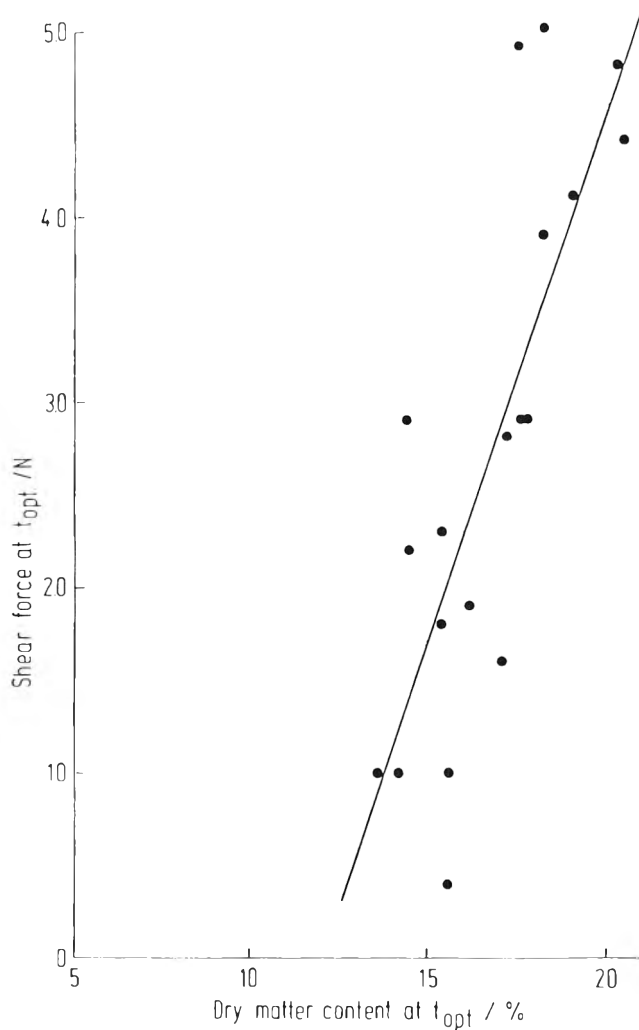
Fig. 3—Correlation between shear force and dry matter content of optimally cooked potatoes (19 varieties, n = 19). F = 0.466 D — 5.255, r = 0.67.
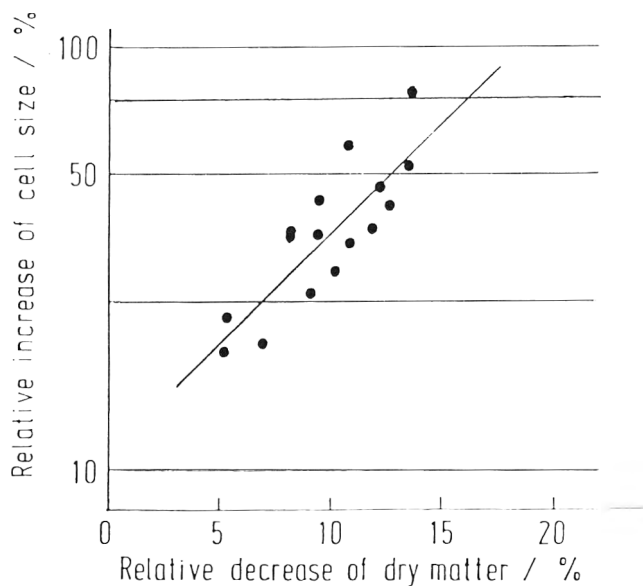

Fig. 4—Correlation between relative increase of cell size and relative decrease of dry matter content at optimal cooking time (16 varieties, n = 16). log C_{rel} = 1.044 + 0.051 D_{rel}, r = 0.81.

REFERENCES

Barrios, E.P., Newsom, D.W., and Miller, J.C. 1963. Some factors influencing the culinary quality of irish potatoes. 2. Physical characters. Am Potato J. 40: 200.

Hadziyev, D. and Steele, L. 1979. Dehydrated mashed potatoes-chemical and biochemical aspects. Adv. Food Res. 25: 55.

Harada, T. and Paulus, K. 1985. Some methods to characterize to cooking behaviour of potatoes. Potato Research (In press).

Harada, T., Tirtohusodo, H., and Paulus, K. 1985. Influence of temperature and time on cooking kinetics of potatoes. J. Food Sci. 50: In press.

Keijbetz, M.J.H. 1974. Pectic substances in the cell wall and the intercellular cohesion of potato tube tissue during cooking. Publikatie 275. Institute for Storage and Processing of Agricultural Produce — IBVL. Centre for Agricultural Publishing and Documentation, Wageningen.

Linehan, D.J., Stooke, C.E., and Hughes, J.C. 1968. The importance of cell size in influencing the texture of the cooked potato. 1. Preliminary observation. Eur. Potato J. 11: 221.

Linehan, D.J. and Hughes, J.C. 1969a. Texture of cooked potato. 1. Introduction. J. Sci. Fd. Agric. 20: 110.

Lienhan, D.J. and Hughes, J.C. 1969b. Texture of cooked potato. 2. Relationships between intercellular adhesion and chemical composition of the tuber. J. Sci. Fd. Agric. 20: 113.

Paulus, K., Heintze, K., Zohm, H., and Fricker, A. 1978. Der Einfluß thermischer Behandlung von Spinat im Temperaturbereich zwischen 100 und 130°C auf den Gehalt an wesentlichen Inhaltsstoffen. 1. Beschreibung der Versuchstechnik bei der Behandlung mit Wasser. Lebensm.-Wiss. u.-Technol. 11: 293.

Reeve, R.M. 1977. Pectin, starch, and texture of potatoes: Some practical and theoretical implication. J. Text. Studies 8: 1.

Warren, D.S. and Woodman, J.S. 1974. The texture of cooked potatoes. A review. J. Sci. Fd. Agric. 25: 129.

Warren, D.S., Gray, D., and Woodman, J.S. 1975. Relationship between chemical composition and break down in cooked potato tissue. J. Sci. Fd. Agric. 26: 1689.

Williams, T.R., 1963. Polysaccharides in potatoes and their effect on the texture of potato products (abstract). Proc. 2nd trien. Conf. eur. Assoc. Potato Res. Pisa, Italy, p. 177.

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times and the cooking behavior to be relatively precisely predicted. Conditions prevailing at optimal cooking time could be explained by cell extension and simultaneous decrease in dry matter content by leaching.

Further correlations were found in this study, such as a correlation between shear force and dry matter content at optimal cooking time, and a correlation between changes in cell size and dry matter content contributed to explaining cooking kinetics and cooking behavior.

Sweetness Evaluation of Mixtures of Fructose with Saccharin, Aspartame or Acesulfame K

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─────────── ABSTRACT ───────────

Some sensorial properties of synthetic sweeteners are limiting factors for use in low calorie soft drinks. By combining synthetic sweeteners with small fructose additions, these limitations can be overcome. Using paired comparison evaluation tests, nonlinear sweetness/concentration relations were established against sucrose, in acidified noncarbonated mineral water, for fructose, saccharin, aspartame and Acesulfame K. In binary combinations with fructose, sweetness additivity was demonstrated, for each of the three synthetic sweeteners. This is in contrast with literature reporting specific synergistic effects. By taking advantage of the high relative sweetness of fructose, low calorie soft drinks containing as little as 2 – 3% sugar could not be distinguished sensorially from traditional sucrose drinks.

INTRODUCTION

THE INCREASING INTEREST for low-calorie and special dietary foods encouraged investigators to develop new synthetic sweeteners. Among them aspartame (aspartyl-phenylalanine methyl ester) and Acesulfame K (3.4-dihydro-6-methyl-1.2.3.-oxathiazine-4-one-2.2-dioxide potassium salt) seem to be the most promising products besides saccharin. Using these sweeteners as the single sweetener in low-calorie foods often results in poor or objectionable sensorial properties. Off- or after-tastes can be detected e.g. the bitter after-taste of saccharin (Larson Powers and Pangborn, 1978a, b; Daniels 1973). Lack of "body" is also a major objection to the incorporation of synthetic sweeteners in low calorie foods.

In order to mask aftertastes and give "body" to the product, mixtures of sugars and synthetic combinations have been developed. In these sweetener combinations specific sweetness properties have been detected and synergism has been claimed (Stone and Oliver, 1969; Yamaguchi et al., 1970; Moskowitz and Klarman, 1975; Hyvönen et al., 1978). Synergistic effects are inferred when the sweetness of a mixture is greater than the sum of the sweetnesses of its components. Evaluation of synergism has been done in different ways and objections can be raised to certain methods described in the literature, e.g. comparison of sweetness results obtained by different methods. Among the sugars used in such combinations fructose is the most interesting because of its highest sweetness. This high sweetness is most striking in low concentration and at low temperatures (Hyvonen et al., 1977a).

The actual purpose of the study was twofold: (1) to establish that the 8 –12% sucrose in traditional recipes could be replaced by combinations of small fructose percentages (1 – 5%) assisted by artificial sweeteners at much lower dosage levels than required in drinks exclusively sweetened with chemicals; (2) to obtain accurate data which can be used to predict sweetness levels matching the sweetness of the sucrose reference drinks.

MATERIALS & METHODS

FRUCTOSE was the product Fructamyl C of NV Amylum (Belgium), sucrose from Suiker Tienen (Belgium), Na-saccharin was purchased from Bayer (Germany), Aspartame from GD Searle & Co (USA) and Acesulfame K from Hoechst (Germany). Citric acid was a product of Merck (Germany). All compounds had a purity in excess of 99%. Spa Reine (Spa Belgium), a noncarbonated mineral water, was used to make the test solution. Cola flavor was purchased from Citrusco (Belgium) and the orange flavor from Chaudfontaine (Belgium).

Solution preparation

All solutions were prepared with mineral water, at least 2 hr before testing. This is necessary with fructose so that it reaches mutarotation-equilibrium (Hyvönen et al., 1977b, c), which has a significant effect on the sweetness of fructose solutions (Shallenberger, 1982). As this equilibrium is affected by temperature, all the tests were carried out at ±8°C. Each solution was prepared on a weight/weight basis and brought to pH 3 with 0.05% citric acid, which is closer to soft drink applications than sweetness evaluation tests at neutral pH.

Taste panel

The panel consisted of 10 highly trained subjects, selected from a larger group according to their performance in sweetness evaluation. All tests were carried out in separate taste booths, airconditioned and equipped with constant illumination. The samples (±20 mL) were swallowed and oral rinsing was performed between two samples. only one sweetener concentration was evaluated per panel session.

Sweetness evaluation experiments

The method used was a modification of the constant-stimulus paired comparison method (Cameron, 1945; Yamaguchi et al., 1970). The sweetness of a test sample at a given concentration (sample D) was compared with a set of three randomised sucrose solutions at different concentrations (sample A, B and C). Three sucrose solutions with respective concentrations of X, X + (X/10) and X − (X/10) seemed to be the most practical number of test samples.

The panel members were first asked to rank the three sucrose concentrations in order of increasing sweetness level. This question was included to check the sweetness evaluation ability of the panel members at each test session. About 80% of the trained panel members were able to rank the reference samples in the correct order. If the sucrose concentration range was decreased an insufficient number of panelists succeeded in correct ranking.

The panel members were then asked to match the test sample D with one of the sucrose concentrations or with a value between two of them.

Using the data given in Fig. 1 to 4, mixtures of fructose with one of the synthetic sweeteners were prepared. The percentages of the respective components were graphically derived in a way that total sweetness should be equal to the sweetness of a 10% sucrose solution. The sweetness of these mixtures was evaluated in a paired comparison test where the test solution was compared to three sucrose solutions with a concentration range from 8 – 12%. Synergism was inferred when the sweetness of a mixture was greater than the summation of the theoretical sweetness values of the components.

The solutions were adjusted to pH 3.0 and the testing temperature was 8°C as for the sweetness evaluation of the single solutions.

Panel members were also asked to comment on possible off-tastes or on varying sweetness responses.

Interpretation of taste results

Only the answers of the panel members who were able to rank the reference sucrose solutions in the right order, were selected for further interpretation. For each acceptable evaluation of the sweetness of the test sample we attributed to this sample a value corresponding to a sucrose concentration with equivalent sweetness. This was done in two ways. First, if the test sample was found to be equal in sweetness to one of the reference sucrose solutions, this sucrose concentration was considered "Equi-sweet." But, if the test sample was ranked between two sucrose concentrations, we attributed to the test sample a value corresponding to the mean of the two sucrose concentrations. As a result of each taste session, which represented 20 replications per sample, we were able to derive an "Equi-sweet" sucrose concentration for every test sam-

ple, by calculating the statistical average of the individual judgements. Relative sweetness is defined as the ratio of the "Equi-sweet" sucrose concentration to the corresponding sweetener concentration. This method takes into account that a panel member can also evaluate the test sample as being equi-sweet with one of the reference solutions and as a result is not forced to rank the test solution between two reference solutions.

Evaluation of low calorie soft drinks

In order to check the results obtained with model systems, two soft drinks, a cola and an orange drink, were formulated to include mixtures of 3% fructose with one of the synthetic sweeteners. The sweetener composition is given in Table 2. The soft drinks were acidified with 0.24% citric acid solution (50% w/w) and contained 0.015% sodium benzoate as a preservative.
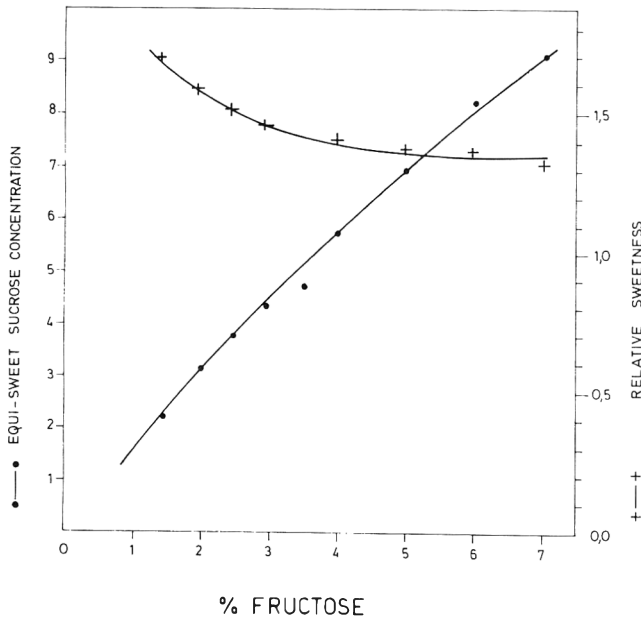
Fig. 1—"Equi-sweet" sucrose concentration and relative sweetness as a function of fructose concentration.
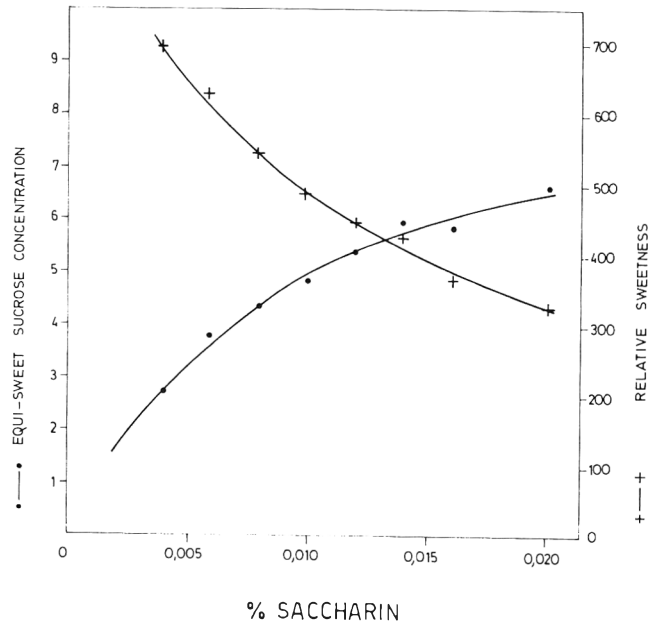
Fig. 2—"Equi-sweet" sucrose concentration and relative sweetness as a function of saccharin concentration.
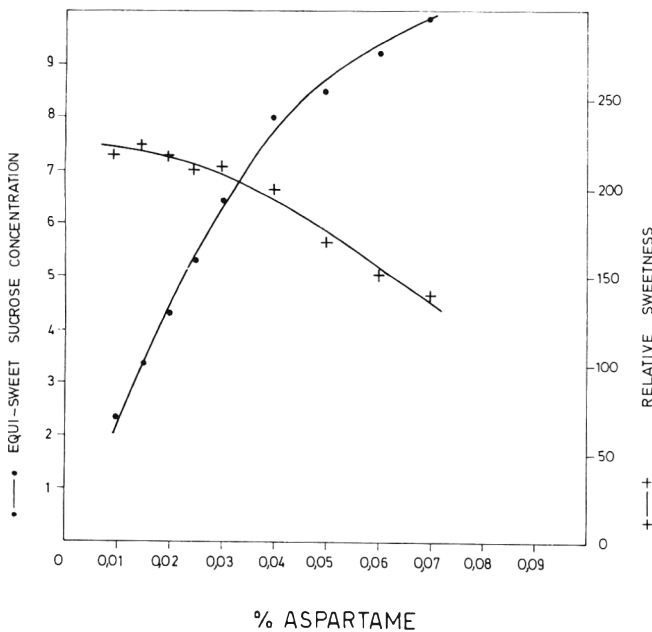
Fig. 3—"Equi-sweet" sucrose concentration and relative sweetness as a function of aspartame concentration.
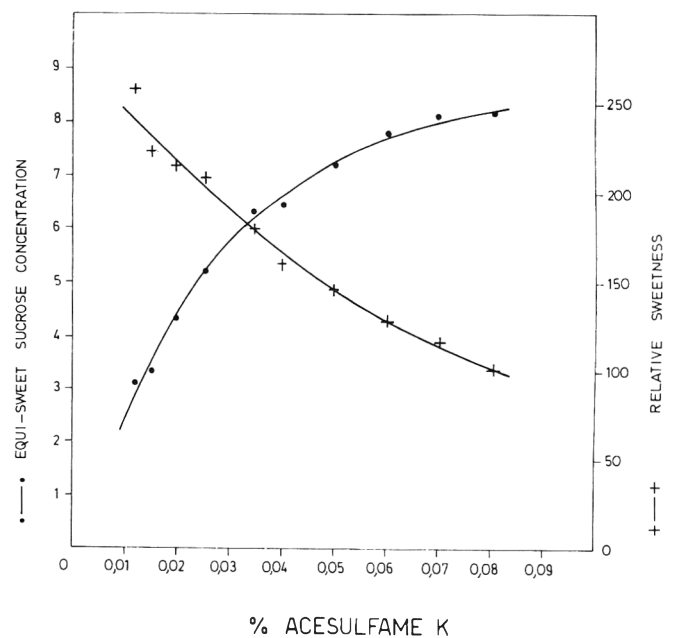
Fig. 4—"Equi-sweet" sucrose concentration and relative sweetness as a function of Acesulfame K concentration.

The reference cola drink was based on 11% sucrose while the reference orange drink contained 9.7% sucrose. The sweetness of the low calorie soft drinks was theoretically matched to the respective reference drinks by calculation on the basis of the data obtained with model systems (Eq. 1 to 4).

The low calorie soft drinks were then evaluated by the taste panel in a triangle test against the reference drink. The panel members were also asked to comment on possible deviating sensorial properties.

RESULTS

SWEETNESS VALUES for fructose and for three artificial sweeteners as a function of their concentration, at pH 3, were obtained using a paired comparison taste panel method. Fig. 1 to 4 represent the average (N = 20) "Equi-sweet" sucrose concentration (ES) and the calculated relative sweetness as a function of the concentration, in the case of four sweeteners evaluated separately (fructose, saccharin, aspartame, Acesulfame-K).

The ES-value expressed a sucrose percentage (w/w) to which the tested sample was equivalent in sweetness. The sweetener concentration is expressed in % (w/w).

The statistical expression of the relation between ES and concentration is given by the following four equations:

$$\text{Fructose: ES} = 1.767 \cdot C_f^{0.848} \quad (r = 0.986) \quad (1)$$

$$\text{Aspartame: ES} = 0.118 \cdot C_a^{0.684} \quad (r = 0.984) \quad (2)$$

$$\text{Saccharine: ES} = 0.451 \cdot C_s^{0.509} \quad (r = 0.994) \quad (3)$$

$$\text{Acesulfame K: ES} = 0.237 \cdot C_k^{0.541} \quad (r = 0.984) \quad (4)$$

This type of relationship was ascertained by applying a linear correlation calculation method to the logarithms of the ES-values and of the corresponding sweetener concentrations.

The graphical representation shows that the relative sweetness (sucrose = 1) was most dependent on the concentration and that the importance of this effect was inversely related to that concentration; the relative sweetness increased with decreasing sweetener concentration. Although this variability was lowest with fructose, it was still appreciable. For instance, when fructose concentration decreased from 7% to 3%, the relative sweetness increased from 1.33 to 1.46. The variability in relative sweetness was most striking with saccharin. Relative sweetness doubled, from 330 to 660, for a decrease in concentration from 0.02% to 0.005%. From these data one can postulate that the sweetness efficiency of these sweeteners is much higher at low concentrations than it is at high concentrations. This is in line with general affinity kinetics, e.g. receptor binding (Freychet 1976; Beidler, 1974).

The results obtained by combining 2.5%, 4% and 5% fructose with saccharin, aspartame or Acesulfame K are given in Table 1. The synergism observed in these experiments did not exceed 10% which is within the accuracy of the evaluation method and therefore statistically nonsignificant. We used an identical sweetness evaluation-method for pure sweetener solutions and for combinations of sweeteners, in contrast to the work of Hyvönen et al. (1978) who used the magnitude estimation method for the sweetness evaluation of single sweetener solutions, and the paired comparison method for the combinations. Hyvönen claimed synergism in fructose saccharin mixtures up to 60%. Our findings are in agreement with Beidler (1974) that the summation of sweetness values obtained by magnitude estimation methods often leads to a kind of synergism which should be defined as "false synergism".

When using paired comparison evaluation methods (Table 1), the algebraic summation of "equi-sweet" concentrations gave a very good prediction of the actual sweetness of mixtures of fructose and a synthetic sweetener such as saccharin, aspartame or Acesulfame K. Only a slight, but nonsignificant difference was detected for the fructose-Acesulfame K mixture.

Thus, when fructose was combined with saccharin, with aspartame, or with Acesulfame K, no synergistic sweetness effects were detected. In fact, as a result of the nonlinear relative sweetness versus concentration relationship (Fig. 1 – 4), each component of a mixture can be used with greater efficiency than either alone at higher concentrations. From this it is obvious that total sweetness of a sweetener mixture can be brought to an optimum. This is comparable with the findings of Weickmann et al. (1969) who stated that synergism was maximal when the components of a mixture contributed about the same amount to its sweetness. With the approach we have adopted one can see that the basis of this phenomenon is not synergism but rather an optimal sweetness efficiency for the different mixture components. For every sweetener component in a mixture there will always be a compromise between relative sweetness and net sweetness contribution.

Table 1 indicates the proportion of panel members who detected off-tastes, using a sucrose solution as reference. We can state generally that off-tastes were detected at relatively high synthetic sweetener concentrations. This was most evident with saccharin because of its pronounced bitter after-taste. In addition to a higher sweetness efficiency, combining fructose with these synthetic sweeteners resulted in a more acceptable sweetener formulation. Possible off-tastes were drastically decreased because of the much lower synthetic sweetener concentrations needed.

Table 1—Theoretical and experimental sweetnesses, the resulting percentage synergism and the proportion of off-tastes detected in a paired comparison test of different sweetener mixtures[a]

Mixture composition	Component sweetness		Theoretical sum	Experimental value	% synergy	Proportion of off-tastes detected
	Fructose	Synthetic sweetener				
2.5% fructose + 0.0130% saccharin	3.8	5.7	9.5	9.5	0	11/17
4 % fructose + 0.0075% saccharin	5.7	4.3	10.0	9.8	−2%	2/16
5 % fructose + 0.0052% saccharin	6.9	3.4	10.3	10.5	+ 2%	2/16
2.5% fructose + 0.025% aspartame	3.8	5.5	9.3	9.4	1%	5/16
4 % fructose + 0.020% aspartame	5.7	4.3	10.0	10.3	3%	1/16
5 % fructose + 0.014% aspartame	6.9	3.2	10.1	10.3	2%	0/16
2.5% fructose + 0.032% Acesulfame K	3.8	5.9	9.7	10.1	4%	10/15
4 % fructose + 0.020% Acesulfame K	5.7	4.3	10.0	10.9	9%	3/15
5 % fructose + 0.012% Acesulfame K	6.9	2.9	9.8	10.0	10%	1/15

[a] Evaluation was done at 8°C and the solutions brought to pH 3.0 by adding 0.05% citric acid.

Applications

Because it is often claimed that certain parameters, e.g., flavors, mouthfeel, clouding agents etc. could bias the results obtained with pure acidified water solutions, commercial soft drink formulations were also submitted to a comparative taste evaluation. The aim was to check whether calculated percentages of combinations of fructose and artificial sweetener attained the sweetness of the all sucrose soft drink, which represented a target sweetness level. As Table 2 illustrates there was a good equivalence between the sweetness of the reference drinks and the sweetness of the three combined drinks, the compositions of which were based on the results obtained with model systems.

Except for the cola drink based on fructose-saccharin all other formulations were found to be indistinguishable by triangle test from the respective reference drinks based on sucrose. For the fructose-saccharin cola drink, a bitter after-taste was detected and some panel members complained of a lack of body effect. In this case a calorie reduction of $\pm 70\%$ was achieved without significantly changing the sensorial properties of the drink. In practice a compromise will have to be made between different factors such as sweetness, quality, calorie content and price.

The possibility of predicting within the investigated concentration ranges the sweetness of any combination of one or more of these four sweeteners leads directly to an easy handling of concrete problems. A manufacturer of, e.g. soft drinks, may wish to know by calculation the amount of artificial sweetener required in addition to a given small percentage of sugars present, in order to attain the sweetness level of the reference product, i.e. the traditional soft drink. A similar situation applies to food legislative tasks, e.g. the actual case of a low calorie soft drink for which limits of use of artificial sweeteners have to be fixed as a function of the RDI/ADI ratio (Real Daily Intake/Acceptable Daily Intake) and of a drastically reduced limit of the authorized amount of sugar.

REFERENCES

Beidler, L.M. 1974. Biophysics of Sweetness. In "Symposium: Sweeteners," p. 10 – 21, (Ed.) G.E. (Ed.). AVI Publishing Co., Westport, CT'
Cameron, A.T. 1945. The relative sweetness of various compounds and of their mixtures. Can. J. Res. 23; Sec. E, 139.
Daniels, R. 1973. Sugar substitutes and enchancers. Noyes Data Corp. Park Ridge, N.J.
Freychet, P. 1976. Review: Interactions of polypeptide hormones with cell membrane specific receptors. Diabetologica 12: 83.
Hyvonen, L., Kurkela, R., Koivistoinen, P., and Merimaa, P. 1977a. Effects of temperature and concentration on the relative sweet-

Table 2—Comparison of mixture sweetened cola and orange drinks with the respective reference drinks based on sucrose. The triangle test was performed at $8^\circ C$

Sweetener mixture	Proportion of correct separations
Cola drink (reference = 11% sucrose)	
3% fructose + 0.018% saccharin	10/16[a]
3% fructose + 0.033% aspartame	4/16
3% fructose + 0.040% Acesulfame K	7/16
Orange drink (reference = 9.7%)	
3% fructose + 0.012% saccharin	9/16
3% fructose + 0.024% aspartame	3/16
3% fructose + 0.026% Acesulfame K	8/16

[a] Difference is statistically significant at the 0.05 probability level.

ness of fructose, glucose and xylitol. Lebensm.-Wiss. und Technol. 10: 316.
Hyvonen, L., Varo, P., and Koivistoinen, P. 1977b. Tautomeric equilibria of D-glucose and D-fructose: Polarimetric measurements. J. Food Sci. 42: 652.
Hyvonen, L., Varo, P., and Koivistoinen, P. 1977c. Tautomeric equilibria of D-glucose and D-fructose: NMR spectrometric measurements. J. Food Sci. 42: 657.
Hyvonen, L., Kurkela, R., Koivistoinen, P., and Ratilainen, A. 1978. Fructose-saccharin and xylitol-saccharin synergism. J. Food Sci. 43: 251.
Larson-Powers, N. and Pangborn, R.M. 1978a. Paired comparison and time-intensity measurements of the sensory properties of beverages and gelatins containing sucrose or synthetic sweeteners. J. Food Sci. 43(1).
Larson-Powers, N. and Pangborn, R.M., 1978b. Descriptive analysis of the sensory properties of beverages and gelatins containing sucrose or synthetic sweeteners. J. Food Sci. 43: 47.
Moskowitz, H.R. and Klarman, L. 1975. The taste of artificial sweeteners and their mixtures. Chem. Senses and Flavor 1: 411.
Schallenberger, R.S. 1982. Intrinsic chemistry of the nutritive Sweeteners. In "Nutritive Sweeteners," p. 7 – 15, (Ed.) G.G. Birch and K.J. Parker. Applied Science Publishers, London and New Jersey.
Stone, H. and Oliver, S.M. 1969. Measurement of the relative sweetness of selected sweeteners and sweetener mixtures. J. Food Sci. 34: 215.
Weickmann, F., Weickmann, H., Fincke, K., Weickmann, F.A., and Huber, B. 1969. Kaloriearmes sussstofhaltiges Zuckeraustauschmittel. German Patent 1,961,769.
Yamaguchi, S., Yoshikawa, T., Ikeda, S., and Ninimiya, T. 1970. Studies on the taste of some sweet substances. Part 2. Interrelationships among them. Agr. Biol. Chem. 34(2): 187.

REFERENCES

Bourne, M.C. 1976. Texture of fruits and vegetables. In "Rheology and Texture in Food Quality," (Ed.) J.M. de Man, P.W. Voisey, V.F. Rasper, and D.W. Stanley. AVI Publishing Co., Westport, CT.
Harada, T. and Paulus, K. 1984. Some methods to characterize the cooking behaviour of potatoes. Potato Research (In press).
Kovacs, E., Vas, K., and Bencze-Böcs, J. 1975. Objective determination of heat treatment requirements of cooked potatoes. Acta Alimentaria 4: 1.
List, D. and Emschermann, B. 1981. Korrelation zwischen sensorischen und instrumentell ermittelten Texturdaten an ausgewählten Lebensmitteln. Gordian 81: 231.
Loh, J. and Breene, W.M. 1981. The thermal fracturability loss of edible plant tissue: pattern and within-species variation. J. Text. Stud. 12: 457.

Paulus, K., Heintze, K., Zohm, H., and Fricker, A. 1978. Der Einfluß thermischer Behandlung von Spinat im Temperaturbereich zwischen 100 udn 130°C auf den Gehalt an wesentlichen Inhaltsstoffen. 1. Beschreibung der Versuchstechnik bei der Behandlung mit Wasser. Lebensm.-Wiss. u. -Technol. 11: 293.
Paulus, K. and Saguy, J. 1980. Effect of heat treatment on the quality of cooked carrots. J. Food Sci. 45: 239.
Paulus, K. and Tirtohusodo, H. 1982. Untersuchungen zur Garungskinetik von Kartoffeln. Ernährungs-Umschau 29: 255.
Sefa-Dedeh, S., Stanley, D.W., and Voisey, P.W. 1978. Effects of soaking time and cooking conditions on texture and microstructure of cowpeas. J. Food Sci. 43: 1832.
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Microstructure of Selected Binary Food Powder Mixtures

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ABSTRACT

Binary mixtures of sugar, citric acid, malic acid, soy protein and starch, after exposure to various relative humidities, were analyzed by Scanning Electron Microscopy (SEM). It is shown that depending on interparticle surface affinity, concentration and relative humidity, the mixtures could be random, partially random, ordered or partially ordered. The type of bridging, between the aggregated particles depended on their chemical species and the availability of surface moisture.

INTRODUCTION

MANY FOOD POWDERS are mixtures of basic ingredients, nutrients, flavoring agents and other materials of specific functionality. During processing, handling and storage it is essential to assure a uniform composition in these mixtures due to nutritional, technological and economical considerations. In order to produce and maintain uniform mixtures it is worth knowing the type of mixture that is created during the mixing process.

According to Weidenbaum (1958) and Williams (1968), among others, a random mixture of freely flowing particles of equal size, shape, and density has the characteristic that: "the probability of finding a particle of a given type A at any point in the mixture is a constant equal to the proportion of that kind of particle in the whole mixture." If the particles are not identical (exception should be made for color) then a partially randomized mixture will be formed.

Hersey (1975), Yip and Hersey (1977b), Thiel et al. (1981) have described what they called "ordered mixtures," where fine particles are adhering onto larger carrier particles. The "order" element in these mixtures (ordered units) is in that the fine particles are not randomly distributed and are most likely to be at the surface of the larger particles forming an aggregate or an agglomerate. Ordered mixing requires particle interaction through adsorption, chemisorption, surface tension, frictional, electrostatic or any other type of adhesion (Hersey, 1975). This is in contrast to a truly random mixture where no cohesive or adhesive forces is of appreciable magnitude (Staniforth, 1981). If the ordered units are not equally sized, a segregation process will occur and a "segregated ordered mixture" will be obtained (Thiel et al., 1981).

In general, there is a limit to the number of fine particles that can adhere to a larger particle. If the remaining fine particles in excess of this quantity, especially if cohesive, can agglomerate with their own kind, they will create a "partial ordered random mixture" (Hersey et al., 1979; Theil et al., 1981).

Egerman and Orr (1983) have questioned the terms "random" and "ordered" in classifying powder mixtures on the grounds that particle interaction and their distribution in the mixture can be due to different physical phenomena.

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They have therefore suggested the term "interactive mixture" be used instead of ordered mixtures, when adhesional phenomena are dominant. "Noninteractive" will replace "random mixture" when all the ingredients are free flowing.

According to Staniforth (1981) a binary mixture of particles having a diameter of 100 microns and above will be mainly random since for this particle size the gravitational force dominates over interparticle electrostatic forces. Mixtures of particles having 40 microns and less tend to be ordered mixtures because at this size range interparticle forces become a decisive factor.

Interparticle surface attraction can also be affected by the presence of moisture through the formation of interparticle liquid bridges. This mechanism can be responsible for the aggregation of particles that do not adhere in a dry state due to the absence of surface forces. Such forces, it ought to be added, can also be affected by atmospheric humidity. The intensity of Van der Waals forces, for example, tend to increase with the humidity while that of electrostatic forces, attractive or repulsive, tends to decrease (Stephenson and Thiel, 1980).

The formation of interparticle liquid bridges requires the presence of a free liquid phase at the particles surface. The source of the liquid is usually moisture from the atmosphere or melting. Recently Downton et al. (1982) analyzed the adherence mechanism during caking and agglomeration of hygroscopic powders and demonstrated that increasing the interparticle liquid surface tension and the interparticle contact time increased the adherence tendency while higher viscosity or larger interparticle distance decreased it.

In recent years Scanning Electron Microscopy (SEM) has become a convenient and useful tool in microstructural analysis of nonfood and food powders and mixtures since it offers the possibility of high resolution and a large field of depth (Johari and Bhattacharyya, 1969; Hollenbach et al., 1982, 1983).

The objective of this work, was to observe the microstructure of selected model food powder mixtures composed of crystalline, proteinaceous and starchy ingredients and to classify them according to their particles capacity to adhere as a result of exposure to various humidity conditions.

MATERIALS & METHODS

Ingredients

Binary mixtures were prepared by using granular sucrose (from a local store), industrial granular citric acid (Miles Laboratories, Inc. Biotech. Products Division, Elkhart, IN), industrial granular malic acid (Alberta Gas Chemicals, Parsippany, NJ), precipitated isolated soy protein (Cargill Inc., Minneapolis, MN), and domestic cornstarch (A.E. Staley Mfg. Co., Oak Brook, IL).

Mixture preparation

Powdered sucrose was obtained by pulverizing the granular sucrose in a laboratory mill (Micro-Mill, Technilab Instruments, Pequanoc, NJ). Before mixing, all the powders (including the powdered sucrose) were sieved for 15 min using a laboratory sieve shaker (Soiltest, Inc., Evanston, IL). For the study reported in this work the fractions used are listed in Table 1.

Prior to mixing, exposure to moisture, and analysis the powders were dried in a vacuum oven (Labline, Inc., Chicago, IL) for 48 hr at 70°C. The dry ingredients of some of the mixtures were exposed to controlled levels of relative humidity at ambient temperature by enclosing the samples in evacuated glass desiccators with silica for $a_w = 0$, water for $a_w = 1$ and saturated salt solutions i.e. LiCl · H_2O for $a_w = 0.11$; $MgCl_2$ · $6H_2O$ for $a_w = 0.32$; $NaNO_2$ for $a_w = 0.65$; KCl for $a_w = 0.85$, as described by Rockland (1975) and Greenspan (1977). The time of exposure was selected to let the mixtures reach equilibrium but, in some cases, mainly at high relative humidities, in order to avoid partial or total dissolution, the time was shorter. The dry or so-treated powders were then mixed at various controlled weight ratios. Few of the dry mixtures were also exposed to the different controlled levels of relative humidity. Every mixture was divided into two equally weighed parts. One part was dried in the vacuum oven for 48 hr at 70°C and the other one was kept wet. After this, the mixtures were sampled and analyzed by Scanning Electron Microscopy (SEM).

SEM sample preparation and observations

Samples were affixed to aluminum stubs with copper tape, coated with approximately 40 nm (400 A) gold palladium and ex-

amined with an ISI Super III-A Scanning Electron Microscope at an accelerating voltage of 15 kV. Images were recorded on Polaroid type, positive/negative film. The components of the mixture were recognized from the micrographs of the pure ingredients and, then, the type of mixture was evaluated.
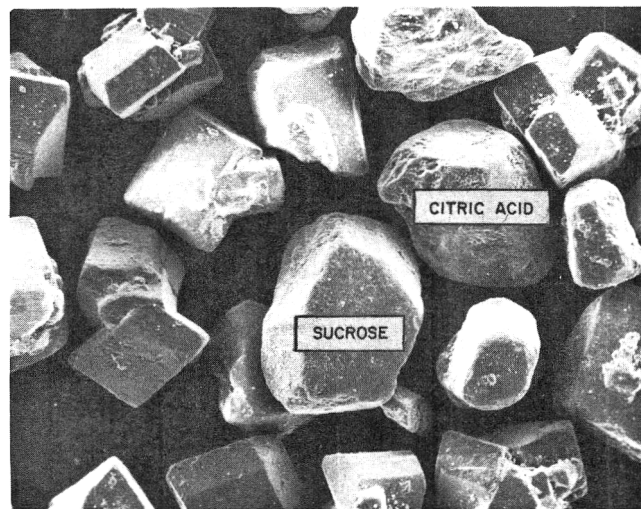
RESULTS & DISCUSSION

THE TYPICAL APPEARANCE of random mixtures composed of free-flowing particles of roughly the same size is shown in Fig. 1 – 3. It is clearly evident from the micrographs that each individual particle is free to move and therefore its location in the mixture is a result of a motion sequence that is controlled by chance only. It can be added that the surface of these particles (i.e. the sucrose and the acids) is not necessarily inert. On the contrary, they can and do attract fines of their own and of other species as shown in Fig. 9 and 11, for example. (See also Hollenbach et al., 1982, 1983). What apparently determined the kind of mixture in this case, therefore, is that the surface forces of the dry particles were insufficient to affect neighboring particles because they were relatively small compared to the weight of the particles.

The typical appearance of an ordered mixture is shown in Fig. 4 – 6. These micrographs illustrate the adherence of the fines to the larger particles surface. The weight fraction of the fines in these samples was such that there was enough surface available for them to adhere to. It is also evident from these micrographs that there was a clear preference to the large particles surface as the site of adherence over the surface of fine particles of the same species.

Fig. 1—Scanning electron micrograph of 1:1 (w/w) dry sucrose and citric acid granules mixture. Note the typical appearance of a random mixture (R.M.).

Table 1—Components used to study mixture types

Ingredients	U.S. Standard mesh	Sieve openings (microns)
Granular sucrose	30 – 50	590 – 297
Powdered sucrose	140 – 270	105 – 53
Citric acid	30 – 50	590 – 297
Malic acid	30 – 50	590 – 297
Soy protein	50 – 70	297 – 210
Corn starch	140 – 200	105 – 74

Fig. 2—Scanning electron micrograph of 1:1 (w/w) dry sucrose and malic acid granules mixture. Note the typical appearance of a random mixture (R.M.).
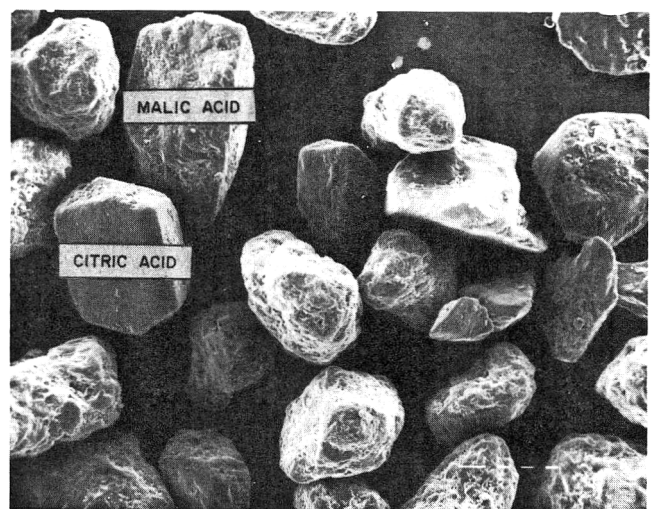

Fig. 3—Scanning electron micrograph of 1:1 (w/w) dry malic acid and citric acid granules mixture. Note the typical appearance of a random mixture (R.M.).

This preference, or surface affinity, was most likely a consequence of the relative strength of liquid bridges in comparison with molecular forces (Rumpf, 1961). Thus, during mixing any aggregate of the same particles (e.g. starch or soy protein) could easily be dispersed while those composed of a particle bound by liquid bridges could not. The case of Fig. 5 is somewhat unique in this respect. But as can be seen from the figure, the fines aggregate, probably bound by liquid bridges themselves, could adhere to the larger particles by forming the same type of bridges. Since the availability of adherence sites at the large particles surface is limited, any excess of fines can either be dispersed randomly or form new aggregates. The appearance of such systems, also known as "partially ordered random mixture," is shown in Fig. 7 – 11. The term refers to the fact that the aggregates, i.e. the "ordered" element, is randomly distributed in the mixture. In other words, if the aggregates are stable enough to be considered as an independent component, i.e. an equivalent to a new particles species, then the mixture appearance is that of a random mixture.

As far as free flowing particles are concerned, there is also another possibility. When the mixture is composed of two noninteractive powders differing in particle size, it can be called "partially random mixture" since an equilibrium is established between the randomization (mixing) and segregation (demixing) processes. An example of its appearance is shown in Fig. 12.

Effects of particle size and water activity

Although this communicaton focuses on the appearance of different types of powder mixtures and not on the mechanisms by which they are formed, a few words ought to be added regarding the two most influencial factors that govern the mixing characteristics of powders. In general, interaction between particles is regulated by the relationship between the strength of the attractive (or repulsive) forces and gravitational forces [i.e. the weight of the affected particle(s)]. Thus, surface attraction can have a negligible effect where only large particles are involved (e.g. granular sucrose), and a decisive effect where fine particles of the same chemical species are involved (e.g. powdered scurose). Such effects are not only evident in the powder microstructure and the appearance of the particles

but also, as previsouly mentioned, in totally altered bulk properties such as density, compressibility and flowability. (The latter is partly a reflection of the ability of particles to overcome the attractive forces.) It is, therefore, clear that the different kinds of "ordered mixtures" are more likely to be formed when at least one of the components has a considerably small size. This, of course is not only a consequence of a smaller particle weight but also of the fact that a larger surface area (especially as a result of grinding, see also Flink, 1983) is associated with higher surface energy, and that the effective interparticle distance is also by far reduced (Rumpf, 1961).

High water activity in this context mainly acts as a means of providing attractive forces in the form of liquid bridges. These are the result of surface dissolution, lique-
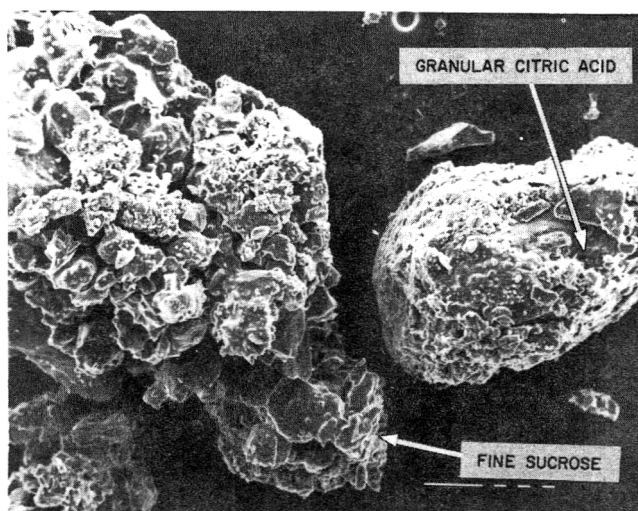

Fig. 5—Scanning electron micrograph of 1:1 (w/w) fine sucrose and granular citric acid mixture after exposure to 65% RH for 24 hr. Note the adherence of the fine sucrose particles to the surface of the large citric acid particles and the typical appearance of an ordered mixture (O.M.).
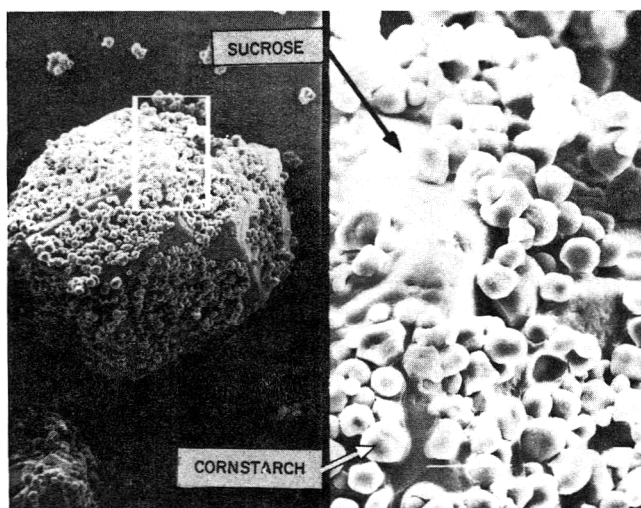

Fig. 4—Scanning electronic micrograph of 9:1 (w/w) granular sucrose and cornstarch mixture after the ingredients were exposed to 100% RH for three hours and then dried. Note the typical appearance of an ordered mixture (O.M.).
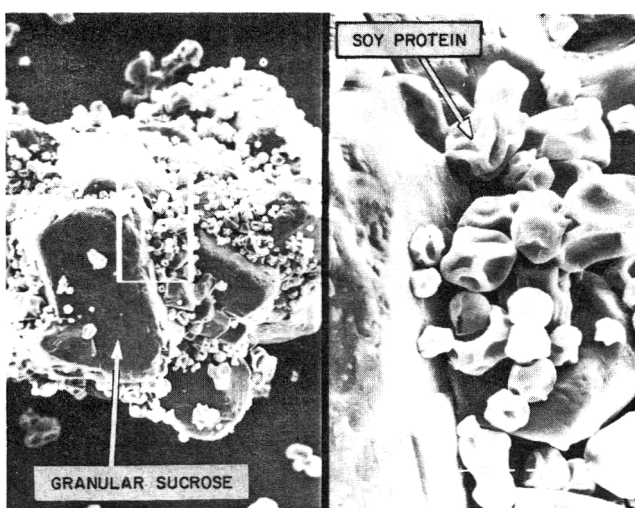

Fig. 6—Scanning electron micrograph of 4:1 (w/w) granular sugar and soy protein mixture after the ingredients were exposed to 65% RH, mixed and then dried. Note the adherence of the soy protein to the surface of the granular sugar (O.M.).

faction or the appearance of a condensed water layer. Surface dissolution can clearly be seen by comparing the sucrose particle surface appearance in Fig. 7 (wet) and Fig. 12 (dry). In such cases, it ought to be added, equilibrium conditions need not to be reached, and as long as enough surface material has been converted to a liquid form, the effect will be practically the same (see Moreyra and Peleg, 1981).

CONCLUSIONS

IT IS EVIDENT that binary food powder mixtures can have different structures and that the latter can be affected, and perhaps controlled, by exposure to moist atmosphere (see Table 2 and Fig. 13). In the reported work, no effort was made to isolate and quantify the effect of the mixture type on the bulk properties of these powders (e.g. density, flowability). Earlier results, however, (e.g. Hollenbach et al., 1982, 1983) indicate that modification of the mixture microstructure can result in considerable changes in bulk properties. It appears, therefore, that the mixture type and the possibility of its control may be a key factor in controlling and perhaps improving powders performance and stability against segregation. This work is only the first stage that was primarily intended to establish the existence of
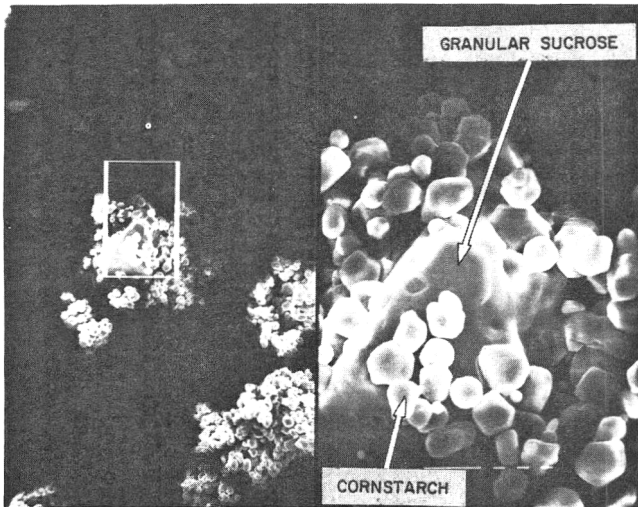
Fig. 7—Scanning electron micrograph of 1:1 (w/w) granular sucrose and cornstarch mixture. This kind of mixture was formed as a result of exposure to high relative humidity (a_w = 1.0) after mixing. Note the adherence of the starch particle to the partially dissolved surface of the sugar particle, the formation of starch agglomerates as a consequence of sucrose surface saturation and the typical appearance of a partially ordered randomized mixture (P.O.R.M.).
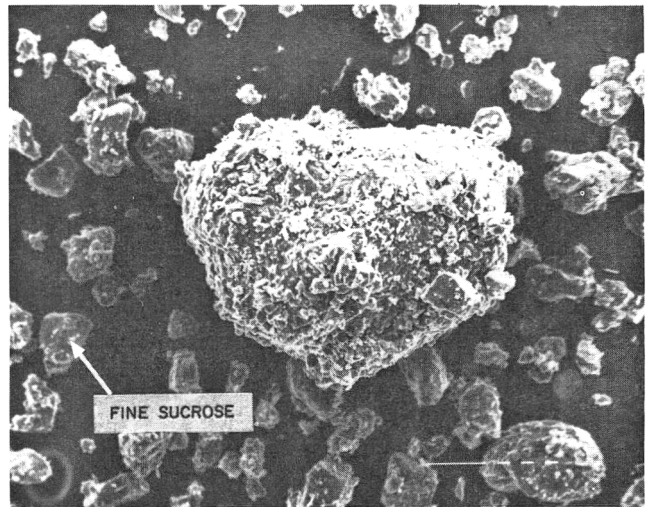
Fig. 8—Scanning electron micrograph of 1:1 (w/w) dry fine sucrose and granular malic mixture. Note the adherence of the powdered sucrose to the surface of the citric acid, th low interaction among fines and the typical appearance of a partially ordered randomized mixture (P.O.R.M.).
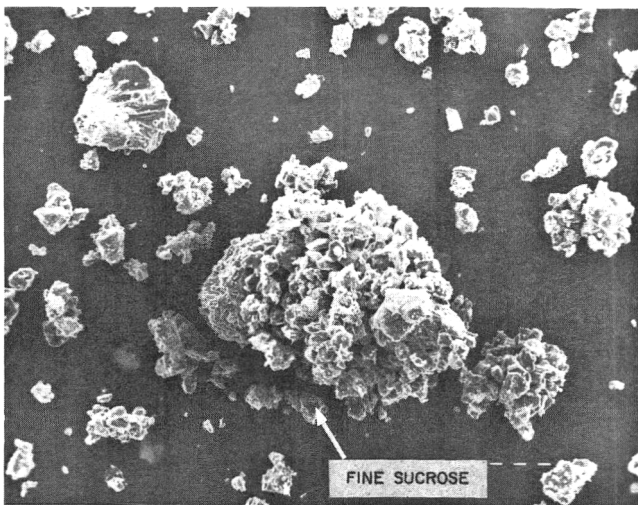

Fig. 9—Scanning electron micrograph of 1:1 (w/w) fine sucrose and granular malic mixture after the ingredients were mixed dry and then exposed to 65% RH. Note the adherence of fines (powdered sucrose) to the surface of the malic acid particles, the formation of fines agglomerates and the typical appearance of a partially ordered randomized mixture (P.O.R.M.).
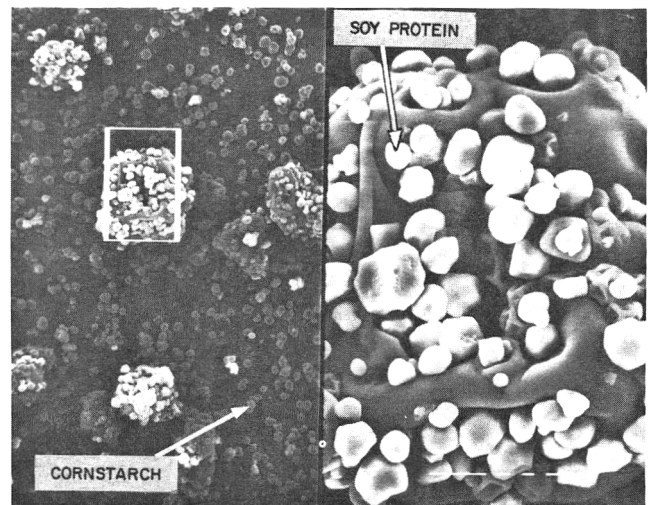

Fig. 10—Scanning electron micrograph of 1:1 (w/w) dry soy protein and cornstarch mixture. Note the adherence of the starch particles to the surface of the soy protein and the free starch particles and the typical appearance of a partially ordered randomized mixture (P.O.R.M.).

different kinds of food powders mixtures. Its results demonstrate that the different kinds indeed exist and that it is worth investigating their quantitative effects on powder flowability and stability against segregation.

REFERENCES

Downton, G.E., Flores-Luna, J.L., and King, J. 1982. Mechanism of sticking in hygroscopic, amorphous powders. Ind. Eng. Chem. Fundam. 21(4): 447.

Egermann, H. and Orr, 1983. Ordered mixtures — Interactive mixtures. Powder Technol. 36: 117.

Flink, J.M. 1983. Structure and structure transitions in dried carbohydrate materials. In "Physical properties of foods, (Ed.) Peleg, M. and Bagley, E.B. AVI Publishing Company Inc., Westport, CT.

Greenspan, L. 1977. Humidity fixed points of binary saturated aqueous solutions. J. Research, National Bureau of Standards 81A(1): 89.

Hersey, J.A. 1975. Ordered mixing: A new concept in powder mixing practice. Powder Technol. 11: 41.

Hersey, J.A. 1979. When disorder leads to order in powder mixing. Powder Technol. 24: 109.

Hersey, J.A., Thiel, W.J., and Yeung, C.C. 1979. partially ordered randomized powder mixtures. Powder Technol. 24: 251.

Hollenbach, A.M., Peleg, M., and Rufner, R. 1982. Effect of four anticaking agents on the bulk characteristics of ground sugar. J. Food Sci. 47(2): 538.

Hollenbach, A.M., Peleg, M., and Rufner, R. 1983. Interparticle surface affinity and the bulk properties of conditioned powders. Powder Technol. 35: 51.

Johari, O. and Bhattacharyya, S. 1969. The application of scanning electron microscopy for the characterization of powders. Powder Technol. 2: 335.

Lai, F., Hersey, J., and Staniforth, J.N. 1981. Segregation and mixing of fine particles in ordered mixture. Powder Technol. 28: 17.
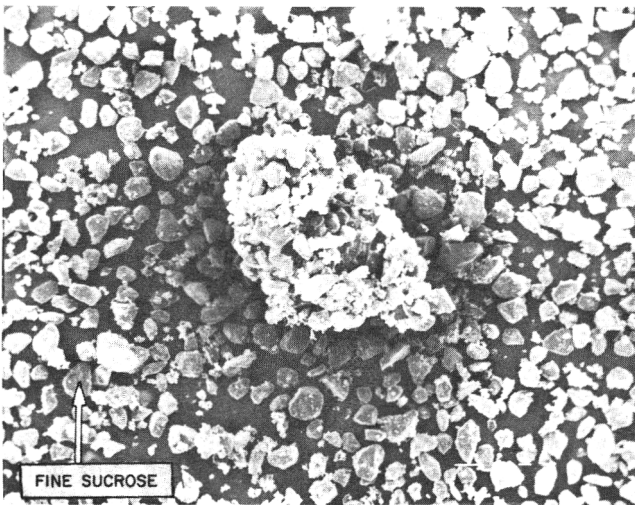
Fig. 11—Scanning electron micrograph of 97:3 (w/w) fine sucrose and granular citric acid. Note the adherence of the sucrose fines to the surface of the citric acid and also, the free sucrose saturation of the carrier surface (R.M.).
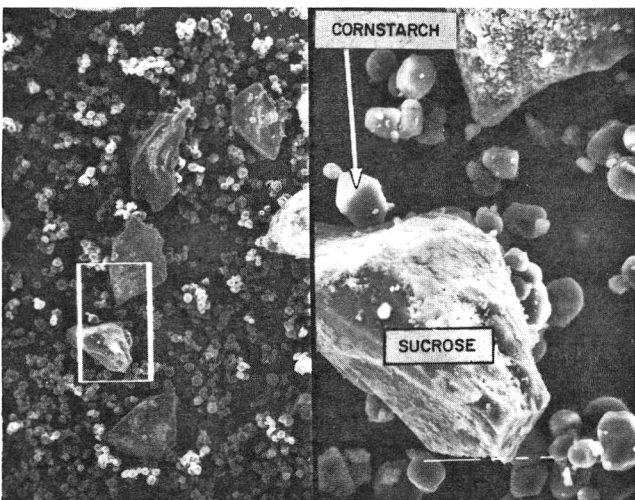
Table 2—Structural classification of selected binary food powder mixtures

Component	Component B	Wt conc [A/B]	Type of mixture[a] Dry	After exposure to moist atm
Fine Sucrose	Granular Malic Acid	50:50	P.O.R.M.[(8)b]	P.O.R.M.[(9)]
	Granular	50:50	P.O.R.M.	O.M.[(5)]
		97:3	P.O.R.M.[(11)]	P.O.R.M.
		95:5	P.O.R.M.	P.O.R.M.
Granular Sucrose	Granular Malic Acid	50:50	R.M.[(2)]	R.M.
	Granular Citric Acid	50:50	R.M.[(1)]	P.O.R.M.
		97:3	R.M.	—
		95:5	R.M.	—
	Cornstarch	50:50	P.R.M.[(12)]	P.O.R.M.[(7)]
		90:10	P.R.M.	O.M.[(4)]
	Soy Protein	50:50	P.R.M.	P.O.R.M.
		80:20	P.R.M.	O.M.[(6)]
Granular Malic Acid	Granular Citric Acid	50:50	R.M.[(3)]	P.O.R.M.
Cornstarch	Soy Protein	20:80	P.O.R.M.	P.O.R.M.
		50:50	P.O.R.M.[(10)]	P.O.R.M.

[a] P.O.R.M. — Partially Ordered Random Mixture; O.M. — Ordered Mixture; P.R.M. — Partially Random Mixture; R.M. — Random Mixture
[b] Numbers in parentheses indicate the figure number in which the particular mixture structure is shown.

Fig. 12—Scanning electron micrograph of 1:1 (w/w) dry granulated sucrose and cornstarch mixture. Note the formation of some small cornstarch agglomerates and the typical appearance of a partially randomized mixture (P.R.M.). There is no interaction among components.
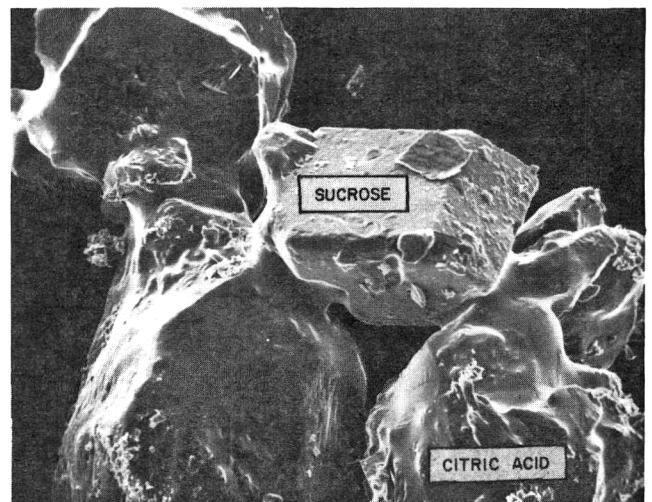

Fig. 13—Scanning electron micrograph of fused aggregates of sucrose and citric acid granules after mixture exposure to 65% RH for 48 hr. In the dry form the two produce a perfect random mixture.

Sensory and Chemical Characteristics of Pork Chops as Affected by Precooking, Curing and Frozen Storage

L. F. MILLER, H. B. HEDRICK, and M. E. BAILEY

ABSTRACT

Four processing procedures were compared as methods of reducing warmed-over flavor (WOF) of pork chops during frozen storage for 84 days. These procedures were (1) oven-broiled chops, (2) chops from loins precooked with no additives, (3) chops from loins cured with 0.5% salt and 40 ppm $NaNO_2$ and precooked, and (4) chops from loins cured with 2% salt and 120 ppm $NaNO_2$ and precooked. Samples were evaluated by the TBA test, Warner-Bratzler shear, hydroxyproline assay and by sensory analysis after frozen storage at $-18°C$. Chops from the three precooked treatments were more tender than oven-broiled chops. Nitrite inhibited WOF development of precooked chops during frozen storage and 40 ppm nitrite was nearly as effective as 120 ppm.

INTRODUCTION

RESEARCH related to precooking or partial cooking of meat prior to frozen storage and subsequent reheating was reported by Watts et al. (1948). Precooked pork products are particularly susceptible to lipid oxidation (Hornstein et al., 1961) and rapidly develop objectionable flavors and odors referred to as "warmed-over flavor" (Tims and Watts, 1958; Dugan, 1961; Younathan and Watts, 1960). These objectionable flavors have been demonstrated to occur rapidly in cooked uncured meats (Younathan and Watts, 1960) and are caused by the oxidation of unsaturated fatty acids catalyzed by iron porphyrins and nonheme iron (Tappel, 1955, Love, 1983).

Several investigators have presented data indicating that precooked pork products were acceptable after reheating (Campbell and Mandigo, 1978; Hope, 1971; Korschgen and Baldwin, 1971; Watts et al., 1948). Hope (1971) reported that pork chops cut from precooked loins and later broiled had more desirable flavor characteristics than fresh broiled pork chops and final preparation represents an advantage to food service establishments and is of particular interest to the pork industry if pork is used as a fast food menu item (Anonymous, 1982).

From the consumer viewpoint there is concern regarding added sodium in processed meat products (Kolari, 1980). The amount of NaCl can be reduced to levels lower than traditionally used in some processed meats and still result in a desirable product (Olson and Terrell, 1981) that may be more stable during storage.

The present study was undertaken to compare sensory and chemical characteristics of oven-broiled pork chops with those of precooked cured and uncured chops after varied periods of frozen storage.

MATERIALS & METHODS

SIXTY-FOUR PORK LOINS were selected on the basis of composite quality score and weight approximately 24 hr post-slaughter. Quality characteristics ranged from a composite score of 2.5 – 3.5

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on the basis of Iowa State Pork Quality Standards (Anonymous, 1969). The two weight groups consisted of an equal number of light (6.3 – 7.7 kg) and heavy weight loins (9.9 – 11.3 kg). The day of selection the loins were individually wrapped in laminated freezer paper, frozen at $-26°C$ and later stored at $-18°C$ for 7 - 28 days.

Eight frozen loins from each weight group were randomly assigned to each of four treatments: (1) frozen loins cut into chops and chops oven-broiled (subsequently referred to as fresh chops); (2) loins precooked with no added seasoning; (3) loins cured with 0.5% NaCl and 40 ppm $NaNO_2$ and precooked; and (4) loins cured with 2% NaCl and 120 ppm $NaNO_2$ and precooked.

Treatment 1 loins were cut while frozen into 2.5 cm thick chops and the frozen chops packaged, identified and stored as described later. Loins assigned to treatments 2, 3 and 4 were removed from the freezer and thawed at $4°C$ for approximately 18 hr. Treatment 3 and 4 loins were then stitch pumped 10% of loin weight (four needle attachment with 2.5 cm needle spacing), with curing solutions containing 5% NaCl and 400 ppm $NaNO_2$, and 20% NaCl and 1200 ppm $NaNO_2$, respectively. Treatment 2, 3 and 4 loins were then stored 18 hr at $2°C$. These loins were subsequently cooked in a smokehouse at $93°C$ to an internal temperature of $66°C$. Smoke generated from hickory sawdust was applied for 1 hr during the cooking process. After removal from the smokehouse, the loins were chilled at $2°C$ for 18 hr. Loins from treatments 2, 3 and 4 were then cut into 2.5 cm thick chops. Twenty center loin chops from each loin were divided into 4 groups of 5 chops each, beginning at the anterior end of the loin. The longissimus muscle portions from the first chop of each group were composited for proximate analysis (AOAC, 1980), sodium determination (AOAC, 1980), and residual collegen analysis using a procedure that combined the collagen separation procedures of Goll et al. (1963) and Paul et al. (1973) and the colorimetric procedure of Bergman and Loxley (1963). The remaining 4 chops from each group were individually packaged in mylar pouches with film penetration characteristics as follows: O_2, 1 cc/6452 cm^2/24 hr at $25°C$; CO_2, 3 cc/6452 cm^2/24 hr at $25°C$; H_2O, 0.3 cc/6452 cm^2/24 hr at $38°C$ and 95% relative humidity. The packaged chops were then frozen at $-26°C$ and stored at $-18°C$ for periods of 0, 28, 56 and 84 days from initiation of study on the frozen loins. At the end of each storage period, chops from treatment 1 were oven-broiled in a natural gas forced air convection oven at $177°C$ to an internal temperature of $74°C$. Precooked chops from treatments 2, 3 and 4 were similarly oven-broiled at $177°C$ to an internal temperature of $43°C$. Sensory, Warner-Bratzler shear, thiobarbituric acid (TBA) (Witte et al., 1970], salt (NaCl) (AOAC, 1980) and residual nitrite (AOAC, 1980) analyses were performed on the longissimus muscle portion of the oven-broiled chops from each processing treatment.

Sensory evaluations were performed by a six member experienced panel. The panelists evaluated samples from all four treatments at each session. An eight point scoring system was used for juiciness, tenderness, flavor and acceptability. The scoring system used for flavor was: 1 = extremely undesirable; 2 = very undesirable; 3 = moderately undesirable; 4 = slightly undesirable; 5 = slightly desirable; 6 = moderately desirable; 7 = very desirable and 8 = extremely desirable. In the case of juiciness, the terms dry and juicy replaced the terms undesirable and desirable that were used for evaluating flavor; for tenderness, the terms tough and tender were used, and for acceptability the terms unacceptable and acceptable were used instead of undesirable and desirable. The panel scoring system for warmed-over flavor (WOF) was as follows: 1 = extremely detectable WOF; 2 = very detectable WOF; 3 = moderately detectable WOF; 4 = slightly detectable WOF; and 5 = no detectable WOF.

Processing losses were calculated from weights obtained at various intervals of processing and storage. The data presented are a summation of cooking, cutting and reheating losses.

The data were analyzed using a split plot and time analysis of variance as outlined by Gill and Hafs (1971). Duncan's New Multiple Range Test was used to separate mean differences where model effects were found to be significant (Snedecor and Cochran, 1979).

RESULTS & DISCUSSION

THE MEAN SQUARES for analysis of variance of various characteristics of pork chops evaluated in this study are presented in Table 1. Processing treatments significantly affected ($P < 0.01$) processing losses, sensory characteristics, Warner-Bratzler shear values, hydroxyproline content, TBA values, protein, moisture, sodium, sodium chloride, and nitrite content of pork chops. The amount of ether-extractable constituents was not related to processing treatment. Type of loin, light vs. heavy weight, had not significant effect ($P > 0.05$) on any of the variables evaluated except sensory panel juiciness score. There were no significant interactions between processing treatments and type of loin. To facilitate presentation of data in Table 2 and in subsequent figures, the data from the two loin weight groups were combined.

Frozen storage significantly affected sensory panel tenderness scores ($P < 0.05$), sensory panel juiciness scores, WOF scores, Warner-Bratzler shear values and TBA values ($P < 0.01$). There were no significant ($P > 0.05$) interactions between processing treatment and storage period, loin type and storage period, or between processing treatment, loin type and storage period.

Mean values for total processing losses for the four pork chop treatment groups are presented in Table 2. The precooked chops processed with 2% salt and 120 ppm nitrite (treatment 4) had the lowest processing losses ($P < 0.05$). The lower processing losses of treatment 4 compared to treatment 3 (0.5% salt and 40 ppm nitrite) can be attributed to the water binding effect of the higher salt level (Wierbicki et al., 1957).

Fresh broiled chops (treatment 1) had higher Warner-Bratzler shear values and lower sensory panel tenderness scores ($P < 0.05$) than chops from the three precooked treatments. Precooking consistently improved tenderness and the addition of salt in the precooked treatments improved tenderness compared to the precooked no salt

Table 1—Mean squares for analysis of variance of characteristics of pork loin chops

Variable	Processing treatment (P) 3	Loin type (L) 1	PXL 3	Replication 56	Storage (S) 3	PXS 9	LXS 3	PXLXS 9	Error 168
Total processing loss	990.7**	321.9	3.2	150.9	184.3	41.3	5.3	9.7	36.8
Sensory panel tenderness	81.8**	0.3	3.7	1.7	2.5*	2.0	0.1	0.6	0.9
Juiciness	33.6**	11.7**	0.9	1.1	4.5**	0.1	0.3	0.6	0.9
Flavor	39.5**	0.1	0.6	1.3	1.5	1.2	0.9	0.7	0.8
Acceptability	45.8**	0.3	0.7	1.5	1.8	1.3	0.4	1.4	0.9
Warmed over flavor	14.7**	0.0	0.2	0.3	2.1**	0.3	0.2	0.2	0.3
Warner-Bratzler shear	249.8**	11.9	6.9	5.9	11.0**	1.4	1.2	1.5	1.3
Hydroxyproline[a]	1.0**	0.1	0.3						0.1
TBA value	355.1**	12.0	6.9	3.5	9.9**	3.5	0.2	1.3	1.9
Crude protein[a]	90.8**	2.2	6.5						2.3
Ether extract[a]	8.8	0.2	3.0						6.0
Moisture[a]	112.1**	7.7	0.3						5.9
Sodium[a]	1.8**	0.0	0.0						0.0
NaCl[a]	14.9**	0.0	0.1						0.1
NaNO$_2$[a]	6066.5**	155.8	185.7						126.1

[a] Degrees of freedom for replication 56.
* Significant ($P < 0.05$); ** Significant ($P < 0.01$).

Table 2—Effect of processing treatment on mean processing losses, shear values, sensory and chemical characteristics of pork chops

	Processing treatment				
	1 Fresh broiled	2 Precooked unseasoned	3 Precooked 0.5% NaCl 40 ppm NaNO$_2$	4 Precooked 2% NaCl 120 ppm NaNO$_2$	SE[e]
Processing loss, %	35.79[a]	36.30[a]	35.57[a]	28.05[b]	0.49
Warner-Bratzler shear, kg/1.27 cm	4.03[a]	2.46[b]	2.37[bc]	2.01[c]	0.09
Hydroxyproline, mg/g	1.93[a]	1.36[b]	1.48[b]	1.45[b]	0.04
Sensory panel:					
Juiciness	3.30[c]	4.23[b]	4.34[b]	5.06[a]	0.13
Tenderness	3.78[b]	5.68[a]	5.89[a]	6.27[a]	0.15
Flavor	4.21[c]	3.78[d]	4.89[b]	5.57[a]	0.14
Acceptability	4.02[c]	3.67[c]	4.77[b]	5.58[b]	0.15
Warmed-over flavor	4.13[b]	3.32[c]	4.04[b]	4.48[a]	0.02
TBA, (mg/kg)	5.66[a]	6.09[a]	1.95[b]	1.67[a]	0.74
Crude protein, %	21.34[c]	26.47[a]	24.06[b]	21.69[c]	0.06
Ether extract, %	5.09[a]	6.28[a]	5.98[a]	4.69[a]	0.31
Moisture, %	72.04[a]	65.89[d]	68.12[c]	70.16[b]	0.94
Sodium chloride, %	0.02[c]	0.01[c]	0.63[b]	2.06[a]	0.02
Sodium, %	0.05[c]	0.05[c]	0.23[b]	0.75[a]	0.004
Nitrite, ppm	0.70[c]	0.38[c]	18.10[b]	41.64[a]	1.40

[a,b,c,d] Values bearing different superscripts on the same line are different ($P < 0.05$)
[e] Standard error of the mean.

treated samples. The lower hydroxyproline content of samples from the three precooked treatments compared to that of fresh broiled samples are consistent with the lower shear values and higher sensory panel tenderness scores for chops receiving these treatments.

Sensory panel juiciness, flavor and acceptability scores were higher ($P < 0.05$) for precooked samples containing salt and nitrite than for fresh-broiled samples. Likewise precooked samples containing no salt or nitrite were rated higher for juiciness desirability than fresh-broiled samples. More detectable WOF was observed for precooked-unseasoned samples than for fresh-broiled and precooked samples containing salt and nitrite. The more desirable flavor scores and less detectable WOF in precooked samples containing salt and nitrite can be attributed to the protective effect of nitrite against lipid autoxidation (Sato and Hegarty, 1971; Bailey and Swain, 1973; Fooladi et al., 1979). Precooked samples processed with 120 ppm nitrite were rated more desirable for flavor and had less WOF than samples processed with 40 ppm nitrite indicating that the higher nitrite level provided more protection against autoxidation. However, the protective effect against autoxidation provided by the 40 ppm level of nitrite suggests that lower levels of nitrite may be used than currently used in processed cured meats. The effect smoke application had on flavor and acceptability scores was not determined. However, since treatment 2, 3 and 4 loins all were smoked, the various differences among these three treatments attributed to salt and nitrite appear valid.

The lower TBA values for samples containing nitrite clearly demonstrate the protective effect of nitrite against autoxidation. In addition, the present study indicates that oxidative changes also occur rapidly in fresh chops during cooking and sample preparation.
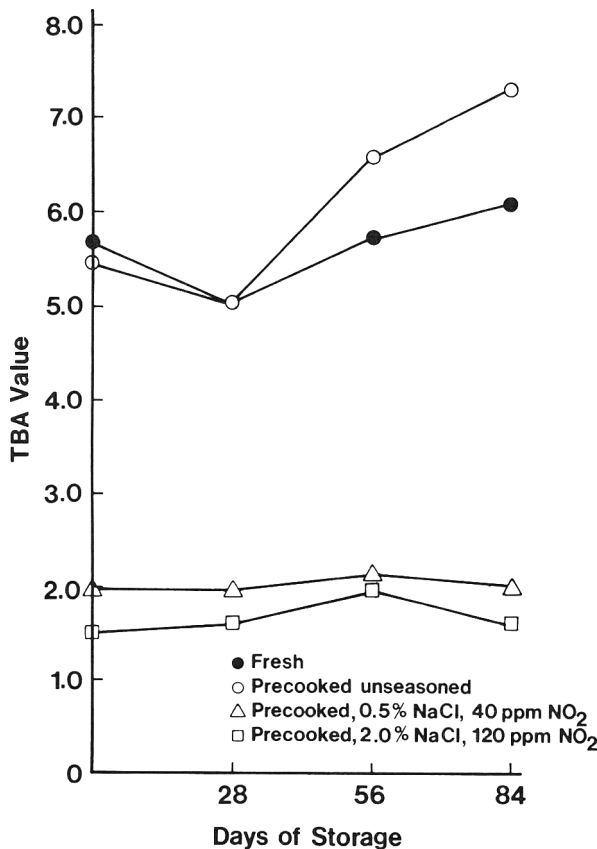
Differences between treatments for protein, ether-extractable constituents and moisture were essentially related to processing losses. Samples from treatment 2, which had the greater processing losses, contained more protein and ether-extractable constituents and less moisture than samples from the other treatments. Sodium chloride, sodium and nitrite levels of fresh-broiled and precooked-unseasoned samples were similar, while the higher levels of these constituents for treatments 3 and 4 were related to the levels of salt and nitrite added during processing.

Data presented in Fig. 1, 2 and 3 illustrate the effect of frozen storage time on TBA values, sensory panel flavor scores and sensory panel WOF scores, respectively. TBA values remained rather constant during the 84 day storage period for the treatments that contained nitrite. TBA values were highest for both the fresh and precooked-unseasoned samples during the period of frozen storage and especially increased for the precooked-unseasoned samples after 28 days of storage.

Flavor scores for the fresh and precooked samples processed with 120 ppm nitrite changed very little during the entire period of frozen storage. Flavor scores for the precooked samples processed with 40 ppm nitrite decreased slightly during frozen storage, and scores for precooked samples processed with no nitrite decreased markedly after 28 days of storage. The changes in flavor scores during the period of storage were significantly related to changes in TBA values ($r = 0.44$).

In general, the sensory panel observed an overall increase in WOF in samples from all processing treatments during the 84 day storage period, the greatest increase

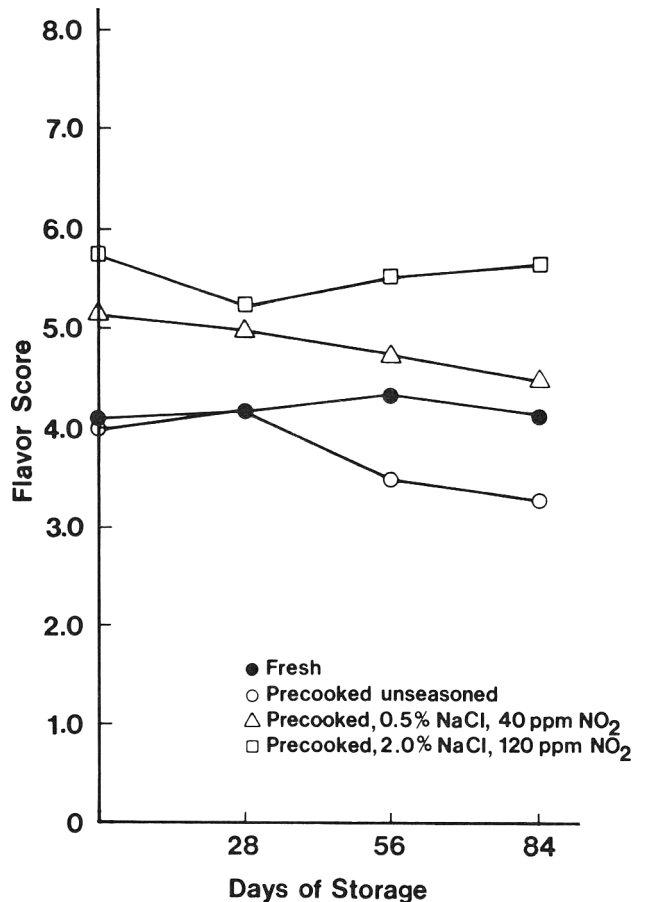Fig. 1—Effect of frozen storage on TBA values (mg/kg) of pork chops.

Fig. 2—Effect of frozen storage on sensory panel flavor desirability scores of pork chops. (Score of 1 = Extremely undesirable; 8 = Extremely desirable).
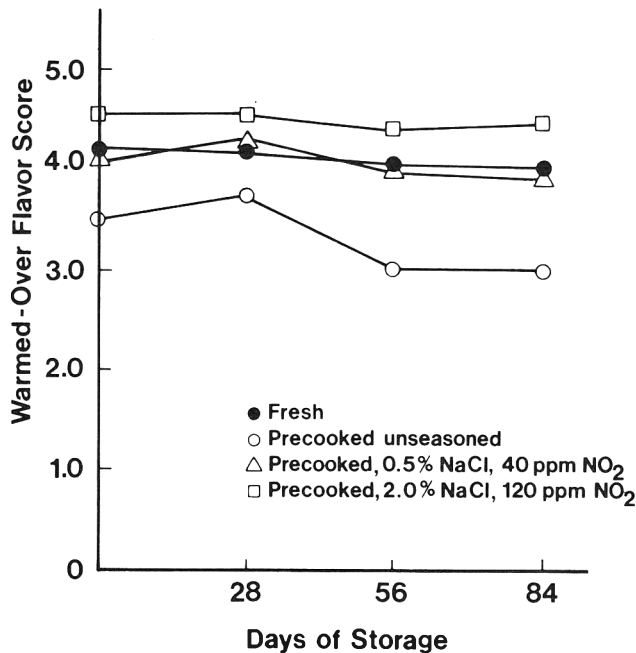
Fig. 3—Effect of frozen storage on warmed-over flavor scores of pork chops (Score of 1 = Extremely detectable; 5 = not detectable).

was observed for precooked samples containing no nitrite. WOF scores were significantly correlated with flavor scores (r = 0.66); as WOF increased, flavor desirability scores decreased.

Results from this study indicate that precooking pork loins in a smokehouse to an internal temperature of 66°C, subsequently cutting the loins into chops and oven-broiling to an internal temperature of 43°C improved tenderness and juiciness compared to nonprecooked chops oven--broiled to an internal temperature of 74°C. Loins containing 2% salt had the lowest processing losses compared to no salt or 0.5% salt. To successfully freeze and store precooked pork chops for potential use by fast food operations, protection from the development of WOF must be provided. Nitrite inhibited WOF development of precooked chops during frozen storage and 40 ppm was nearly as effective as 120 ppm.

REFERENCES

Anonymous. 1969. Standards for Pork Color, Firmness and Marbling. PM-452. Iowa State Univ., Ames, IA.
Anonymous. 1982. Including meat in reducing diets. National Live Stock and Meat Board Reports. May.
AOAC. 1980. "Official Methods of Analysis," 13th ed. Assoc. Official Agr. Chemists, Washington, DC.
Bailey, M.E. and Swain, J.W. 1973. Influence of nitrite on meat flavor. Proc. Meat Ind. Res. Conf. 29: 46.
Bergman, I. and Loxley, R. 1963. Two improved and simplified methods for spectrophotometric determination of hydroxyproline. Anal. Chem. 35: 1961.
Campbell, J.F. and Mandigo, R.W. 1978. Properties of restructured pork patties as affected by cooking method, frozen storage and reheating method. J. Food Sci. 43: 1648.
Dugan, L.R. 1961. Development and inhibition of oxidative rancidity of foods. Food Technol. 15: 10.
Fooladi, M.H., Pearson, A.M., Coleman, T.H., and Merkel, R.A. 1979. The role of nitrite in preventing development of warmed-over flavour. Food Chem. 4: 283.
Gill, J.L. and Hafs, H.D. 1971. Analysis of repeated measurements of animals. J. Anim. Sci. 33: 331.
Goll, D.E., Bray, R.W., and Hoekstra, W.G. 1963. Age-associated changes in muscle composition: The isolation and properties of a collagenous residue from bovine muscle. J. Food Sci. 28: 503.
Hope, W.D. 1971. Broiled pork chops as influenced by precooking. M.S. thesis, Univ. of Missouri.
Hornstein, I., Crowe, P.F., and Heimberg, M.J. 1961. Fatty acid composition of meat tissue lipids. J. Food Sci. 26: 581.
Kolari, O.E. 1980. Salt dietary concern. Proc. Meat Ind. Res. Conf., p. 89.
Korschgen, B.M. and Baldwin, R.E. 1971. Palatability of boneless fresh pork ham and leg of lamb prepared by interrupted cooking prior to frozen storage. J. Food Sci. 36: 756.
Love, J.D. 1983. The role of heme iron in the oxidation of lipids in red meats. Food Technol. 37: 117.
Olson, D.G. and Terrell, R.N. 1981. Sensory properties of processed meats using various sodium salt substitutes. Proc. Meat Ind. Res. Conf., p. 26.
Paul, P.C., McCrae, S.E., and Hofferber, L.M. 1973. Heat-induced changes in extractability of beef muscle collagen. J. Food Sci. 38: 66.
Sato, K. and Hegarty, G.R. 1971. Warmed-over flavor in cooked meats. J. Food Sci. 36: 1098.
Snedecor, G.W. and Cochran, W.G. 1979. Statistical Methods. The Iowa State Univ. Press, Ames.
Tappel, A.L. 1955. Unsaturated lipid oxidation catalyzed by hematin compounds. J. Biol. Chem. 217: 721.
Tims, M.J. and Watts, B.M. 1958. Protection of cooked meats with phosphates. Food Technol. 12: 240.
Watts, B.M., Peng, D., and Kline, E.A. 1948. Precooking pork for freezing storage. J. Home Economics 40: 579.
Wierbicki, E., Cahill, V.R., and Deatherage, F.E. 1957. Effect of added NaCl, KCl, CaCl$_2$, MgCl$_2$ and citric acid on meat shrinkage at 70°C and of added NaCl on drip loss after freezing and thawing. Food Technol. 11: 74.
Witte, V.C., Krause, G.F., and Bailey, M.E. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. J. Food Sci. 35: 582.
Younathan, M.T. and Watts, B.M. 1960. Oxidation of tissue lipids in cooked pork. Food Res. 25: 538.
Ms received 7/23/84; revised 9/20/84; accepted 10/16/84.

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Moreyra, R. and Peleg, M. 1981. Effect of equilibrium water activity on the bulk properties of selected food powders. J. Food Sci. 46(6): 1918.
Rockland, L.B. 1960. Saturated salt solutions for static control of relative humidity between 5 and 40°C. Anal. Chem. 32(10): 1375.
Rumpf, H. 1961. "The Strength of Granules and Agglomerates in Agglomeration," (Ed.) W.A. Krepper. Industrial Publisher, New York.
Staniforth, J.N. 1981. Total mixing. Int. J. Pharm. Tech & Prod. Mfr. 2(1): 7.
Stephenson, P.L. and Thiel, W.J. 1980. The effect of humidity on the production of ordered mixtures. Powder Technol. 25: 115.
Thiel, W.J., Lai, F., and Hersey, J.A. 1981. Comments on "Suggestions on the nomenclature of powder mixtures." Powder Technol. 28: 117.
Thiel, W.J. and Stephenson, P.L. 1982. Assessing the homogeinity of an ordered mixture. Powder Technol. 31: 45.
Weidenbaum, S.S. 1958. Mixing of solids. In "Advances in Chemical Engineering," Vol. 2, (Ed.) Drew, T.B. and Hoopes, J.W. Jr. Academic Press Inc., New York.

Williams, J.C. 1968. The mixing of dry powders. Powder Technol. 15: 237.
Williams, J.C. 1976. The segregation of particulate materials. A review. Powder Technol. 15: 245.
Yip, C.W. and Hersey, J.A. 1977a. Segregation in ordered powder mixtures. Powder Technol. 16: 149.
Yip, C.W. and Hersey, J.A. 1977b. Perfect powder mixtures. Powder Technol. 16: 189.
Ms received 7/23/84; revised 9/17/84; accepted 10/2/84.

Microbial Flora of Lamb Carcasses Stored at 0°C in Packs Flushed with Nitrogen or Filled with Carbon Dioxide

F. H. GRAU, I. J. EUSTACE, and B. A. BILL

──────────── ABSTRACT ────────────

Two treatments to effect low-oxygen conditions for chilled storage of lamb carcasses were compared. Carcasses were placed in bags of low gas permeability and either flushed with nitrogen or filled with carbon dioxide. Carbon dioxide significantly reduced the growth of gram-negative oxidative and fermentative bacteria, *Brochothrix thermosphacta*, and lactic-acid bacteria both on surface tissues and in the purge fluid (weep) from the carcasses. The mean pH of the purge fluid from both sets of carcasses was 6.3. When primal cuts prepared from stored carcasses were held in air at 5°C, development of off-odors was slower for the carcasses that had been stored in carbon dioxide. While the aerobic spoilage flora of loins from unstored carcasses was composed mainly of *B.thermosphacta* and oxidative gram-negative bacteria, this latter group became a smaller component of the spoilage flora of the loins the longer the carcasses had been stored.

INTRODUCTION

WHEN PRIMAL CUTS of lamb are vacuum-packaged and stored at 0-2°C, they remain unspoiled for 6 wk (Patterson and Gibbs, 1978; Shaw et al., 1980). However, on opening packs stored for such a period, the primals have only a short aerobic storage life at 5°C (Shaw et al., 1980). While there are economic advantages in vacuum-packaging lamb carcasses, the large headspace formed by the abdominal and chest cavity poses a potential problem. This relatively large headspace could lead to a higher oxygen and a lower carbon dioxide content than is normally found in vacuum-packaged cuts of meat, and consequently a shorter shelf-life. Furthermore, most of the surface of lamb carcasses is composed of fatty or connective tissue with little exposed lean so that oxygen is likely to be consumed more slowly than occurs with vacuum-packaged primal cuts of meat. The ecological environment of packaged lamb carcasses therefore differs from that of packaged primals.

Flushing with an oxygen-free gas is a means of reducing the amount of oxygen in the headspace. Carbon dioxide would appear to be an ideal gas to use as a flushing gas since atmospheres high in carbon dioxide have been shown to extend the shelf-life of veal, beef, pork, and chicken (Sander and Soo, 1978; Enfors et al., 1979; Seideman et al., 1979; Hess et al., 1980; Blickstad et al., 1981; Erichsen and Molin, 1981). High carbon dioxide concentrations inhibit, in apparently decreasing order of effectiveness, the growth of gram-negative oxidative and fermentative bacteria, *Brochothrix thermosphacta*, and lactic-acid bacteria. The relatively large headspace of packaged carcasses can serve as a gas reservoir to help maintain a high carbon dioxide tension within the pack.

In this study lamp carcasses were vacuum-packaged after flushing the headspace with nitrogen, and other carcasses were packaged in the presence of carbon dioxide. The objectives were to examine the effect of the two gas treatments on (1) the numbers and types of microbial flora that grew at 0°C on the carcasses and in the accumulated purge fluid (weep); (2) the development of off-odors from the packaged carcasses; (3) the development of off-odors at 5°C in air from primal cuts prepared from the stored carcasses; and (4) the composition of the micro-flora of one of these primal cuts (i.e. the loin) when spoiled aerobically.

MATERIALS & METHODS

Meat packaging

Thirteen lamb carcasses (without kidneys) were obtained 24 hr after slaughter from a commercial abattoir. The carcasses had been chilled in air at 0-4°C and weighed 13.1-14.7 kg (mean 14.0). The brisket was cut through from the abdominal opening to the neck. The forelegs and neck were cut from each carcass, tied together and placed in the chest cavity. The back-bone was cut through between the sixth lumbar and first sacral vertebra. The hindquarter was then folded so that the hindlegs of the carcass were inserted through the abdominal opening into the chest cavity. "Bone-gard" (W.R. Grace, Melbourne, Australia) was placed over the butt of the neck, the foreleg joints, the brisket, and over the exposed vertebral column to prevent bone puncturing the packaging bags. The "telescoped" carcasses were then individually packed in plastic bags (92 cm × 46 cm; Barrier Bag, T gauge; W.R. Grace). The bags were formed from film composed of a layer of polyvinylidene chloride coated on both sides with ethylene-vinyl acetate copolymer. The nominal oxygen transmission rate was about 35 mL/m² per 24 hr per 101 kPa measured at 25°C and 75% relative humidity.

The carcasses were selected at random and allotted to two treatments. Six of the packed carcasses were evacuated (to 75 kPa) and 5L of high purity nitrogen (Commonwealth Industrial Gases, Brisbane, Australia) added. The evacuation and nitrogen-flush cycle was repeated three times. The packs were then evacuated to 75 kPa and clip sealed (N_2-packs). Seven carcass-packs were evacuated and flushed three times with high purity food grade carbon dioxide (<0.02% oxygen), evacuated to 75 kPa, and finally 5L of carbon dioxide was added to each of the packs before they were clip sealed (CO_2-packs). Evacuation, gas-flushing, and clip-sealing were done using a nozzle-type vacuum-packaging machine (Cryovac, model VS 2109; W.R. Grace) which was modified by the addition of a gas line into the nozzle, and of a rubber sleeve (7 mm thick) and clamp over the nozzle (to reduce air leakage during gas flushing). Only the clipped end of the bag was heat shrunk by immersion for 1 sec in water at 90°C. The packaged carcasses were stored for up to 10 wk in a room maintained at 0-1°C.

Gas analysis

Before opening, 1 mL of gas was removed from each pack by a gastight syringe (Dynatech Precision Sampling Corp., Baton Rouge, LA) inserted through a patch of cured silicone sealing compound (Silastic 732 RTV, Dow-Corning, Australia) fixed to the film with a keying adhesive (Contact Bond Cement, Bostik, Australia). The percentage of nitrogen, oxygen and carbon dioxide was measured using a Fisher Hamilton Model 29 gas partitioner (Fisher Scientific Co., Pittsburgh, PA).

pH measurement

The pH of the M.longissimus dorsi muscle was measured on each of the carcasses before packaging by insertion of a combination electrode (Philips C64/1) into the cut muscle exposed when the back-bone was severed before "telescoping." Purge fluid was warmed to room temperature and its pH measured with a Radiometer TTT 1C pH meter fitted with a GK 2302 C Radiometer combination electrode.

Sampling of carcass-packs, and aerobic storage of primal cuts

Two carcasses (one from each gas treatment) were removed from storage after 1 hr (zero time), and after 2,4,6,8 and 10 wk. At each sampling time, after gas samples were taken, the packs were opened, examined immediately for odor, and microbiological samples of the carcass and purge fluid were taken. The purge was collected and its volume measured.

The carcasses, removed from the packaging material, were allowed to hang at 10°C for 1 hr when they were again appraised.

The carcasses were then each broken into six portions—forequarters, loins, and hindlegs. These primal cuts were placed in individual polyethylene bags (low density polyethylene, 0.035 mm thick, oxygen transmission rate about 4500 mL/m² per 24 hr), and stored at 5°C. The primals were examined twice daily for off-odor by a five-membered panel and scored on a 9-point hedonic scale. When the loins were considered to have a moderate to strong off-odor, they were microbiologically sampled.

Microbiological sampling

Seven sites on each carcass were sampled by excising 2 samples (each 5 cm² × ca 4 mm deep) from each site. For zero time samples (carcasses sampled 1 hr after packaging), four 5 cm² samples were taken from each site. Three sites were from the outside of the carcass (i.e. in contact with the packaging film): brisket, rump and mid-back. Four sites were from inside the body cavity of the telescoped carcass: abdominal wall near the diaphragm, renal area, inside surface of the hindlegs, and the forelegs and neck.

From the aerobically stored loins, the three sites sampled were muscle from the sacral end of the loin, fatty tissue on the outer surface, and connective tissue in the abdominal cavity. For each site sample, a 5 cm² sample was excised from each of the two loins. To each 10 cm² (or 20 cm²) sample 95 mL of 0.1% peptone water was added and the samples treated for 1 min with a Colworth Stomacher, Model 400 (A.J. Seward and Co. Ltd., London, England). Appropriate dilutions were made in 0.1% peptone water. Aliquots (0.1 or 0.2 mL) were spread on the surface of duplicate plates of APT agar (Difco); violet red bile agar (VRB; Oxoid); MRS (de Man, Rogosa, Sharpe) agar (Oxoid); streptomycin thallous acetate actidione agar (STAA; Gardner, 1966); oxytetracycline glucose yeast extract agar (Mossel et al., 1962) modified by the addition of 100 μg/mL streptomycin (OSGY); 0.8% peptone (Oxoid) mineral salts agar (Grau, 1983); and tryptone soya agar (Oxoid) supplemented with 0.5% yeast extract (Oxoid) and 0.2% glucose (TYSG).

Plates were incubated aerobically at 25°C and counted at 2,3 and 4 days. An additional set of MRS agar plates was incubated anaerobically (Oxoid anaerobic system) at 25°C for 5 days. Plates of TYSG and peptone-salts agars were also incubated aerobically at 0°C and counted after 21 days. From each medium up to 10 colonies of each colonial type were isolated onto TYSG, and examined for their growth responses on MRS, STAA and VRB at 25°C, and on TYSG and APT at 5°C. The isolates were further examined for catalase, oxidase (Collins and Taylor, 1967), motility, morphology, gram reaction (Bruck, 1982), oxidative-fermentative utilization of glucose (Hugh and Leifson, 1953), arginine dihydrolyase (Thornley, 1960), DNAse (Bacto-DNase test agar, Difco), H₂S production from cysteine (lead acetate agar, Difco, modified by replacing thiosulfate with 0.02% L-cysteine HCl). Isolates were identified as lactic-acid bacteria, B.thermosphacta, yeasts, Enterobacteriaceae, Aeromonas spp., Pseudomonas spp., Alteromonas spp., moraxella- or acinetobacter-like organisms. After colonies had been isolated from peptone-salts agar plates, the plates were flooded with oxidase reagent (Edwards and Ewing, 1972) to obtain an estimate of the count of oxidase-positive bacteria.

RESULTS

Gas analysis of packaged carcasses

The gas composition in three carcass-packs was measured 1 hr after packaging. The nitrogen-packed carcass contained 0.4% oxygen and 99.2% nitrogen. Two carcasses packed with 5L of carbon dioxide contained 0.4 and 0.6% oxygen, 1.6 and 2.5% nitrogen, and 98 and 97% carbon dioxide. In the CO₂-packs, the film became tight around the carcasses after 1 day of storage at 0°C, presumably because much of the carbon dioxide dissolved in the tissue.

Fig. 1 shows the carbon dioxide concentration in the gas phase in the carcass packs during storage. In the N₂-packs, there was a slow increase in carbon dioxide to reach 16% in 10 wk. In the CO₂-packs, the carbon dioxide concentration fell rapidly to stabilize at around 40% at 4 wk. In both sets, most of the residual gas was nitrogen. The oxygen content was 0.5–1%.

Purge fluid from packaged carcasses

For carcasses stored in nitrogen, the mean volume of purge fluid was 0.7% (range 0.2–0.9%) of the carcass weight. The purge was 2.0% (range 1.3–3.4) for carcasses stored in carbon diox-
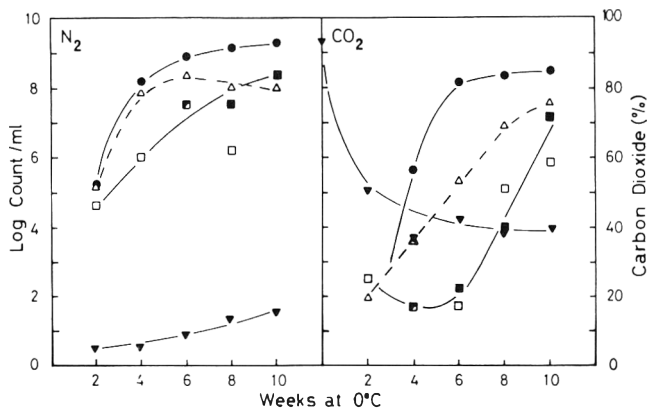

Fig. 1—Growth of psychrotrophic microorganisms in purge fluid, and the carbon dioxide concentration in the head-space of packaged carcasses stored at 0°C: N₂, packs flushed with nitrogen; CO₂, packs containing 5L of added carbon dioxide; ●——●, lactic-acid bacteria; △-----△, B.thermosphacta; □——□, gram-negative oxidative bacteria; ■——■, gram-negative fermentative bacteria; ▼——▼, percent carbon dioxide.

ide. Packaging in carbon dioxide significantly increased the amount of purge (p<0.05; t test). Most of the purge accumulated in the body cavity.

The mean pH of the purge fluid for both sets of carcasses was high (pH 6.3; range 6.02–6.45) even though the pH of the longissimus dorsi muscles at the time of packaging was not (mean pH 5.7; range 5.52–5.90).

The purge contained a large psychrotrophic bacterial population (Fig. 1). For instance, in the N₂-packs, lactic-acid bacteria reached a count of more than 10⁹/mL, and both B.thermosphacta and fermentative gram-negative bacteria reached more than 10⁸/mL. However, there were considerable differences in the microbial growth in the purge from the N₂-and CO₂-packs. In the N₂-packs, lactic-acid bacteria and B.thermosphacta both reached counts of ca 10⁸/mL in 4 wk. In the CO₂-packs, the count of lactic-acid bacteria was 10⁸/mL in 6 wk, and it took 10 wk for the B.thermosphacta count to reach 4 × 10⁷/mL. The growth of the gram-negative flora was also much slower in the purge from the CO₂-packs. After 6 wk storage, the gram-negative count in the purge from the N₂-packs was ca 4 × 10⁷/mL, and from the CO₂-pack was a little over 100/mL. In both types of pack, fermentative gram-negative bacteria (Enterobacteriaceae and Aeromonas spp.) tended to dominate the gram-negative flora at later stages of storage. Up to 4 wk storage, the aerobic gram-negative flora was a mixture of pseudomonads, moraxella- and acinetobacter-like organisms. In the purge from later storage times, alteromonads were commonly detected along with pseudomonads and moraxella-like organisms. Psychrotrophic yeasts were occasionally detected in the purge fluid but never at a count above 200/mL.

Microbial flora on the surface tissue of packaged carcasses

Two carcasses were sampled 1 hr after packaging. The mean aerobic mesophilic count (TYSG incubated at 25°C) of the seven sites on each of these two carcasses was 3.8 × 10³/cm². The psychrotrophic count (TYSG incubated at 0°C) was too low to estimate accurately since eight of the 14 samples gave counts of ≤ 25/cm². The psychrotrophic organisms detected were moraxella-, acinetobacter-like bacteria, and B.thermosphacta.

Fig. 2 shows the effect of storage time on the mean log count of the four major groups of psychrotrophic bacteria on the seven carcass sites. The numbers of bacteria/cm² of surface tissue were considerably lower than the viable count/mL of purge fluid. The growth on the carcasses of the lactic-acid bacteria, B. thermosphacta, and the gram-negative oxidative and fermentative bacteria was slower in the CO₂-packs. On carcasses in the N₂-packs, lactic-acid bacteria and B.thermosphacta were present in approximately equal numbers throughout storage. On the other hand, in the CO₂-
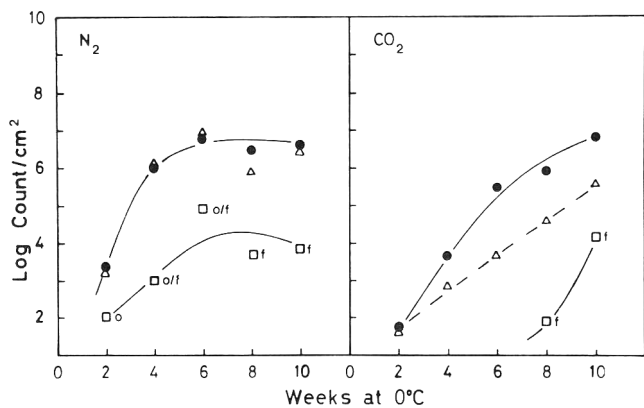
Fig. 2—Growth of psychrotrophic microorganisms on the surface tissue of packaged carcasses stored at 0°C: N_2, packs flushed with nitrogen; CO_2, packs containing 5L of added carbon dioxide; ●——●, lactic-acid bacteria; △-----△, B.thermosphacta; □——□, gram-negative bacteria; O, preoominantly oxidative, f, predominantly fermentative gram-negative bacteria. Each datum point is the mean of the log counts/cm² of 7 sites per carcass.

Fig. 3—Aerobic spoilage (off-odor) at 5°C of primal cuts prepared from carcasses stored at 0°C in nitrogen (△) or carbon dioxide (o): bar, range of times to off-odor; △ and o, time of microbiological sampling of loins.

packs, B.thermosphacta grew more slowly than the lactic-acid bacteria. The growth of the gram-negative bacteria was very strongly inhibited in the CO_2-packs. Fermentative organisms (Enterobacteriaceae and Aeromonas spp.) were the predominant gram-negative bacteria on carcasses stored for 8 and 10 wk. At this stage of storage gram-negative oxidative types (alteromonads, pseudomonads, and moraxella-like organisms) were detected on some carcass sites, but in lower numbers than the fermentative types.

For carcasses stored in the N_2-packs, the counts of lactic-acid bacteria and B.thermosphacta on the foreleg and neck samples were about 10-fold higher than the mean counts from the o:her six tissue samples. No consistent differences were apparent in the counts of these organisms among the other six carcass sites.

For carcasses stored in the CO_2-packs, the counts of the lactic-acid bacteria and of B.thermosphacta were again consistently highest on the foreleg and neck. In addition, there was also a consistent difference in the counts of B.thermosphacta between samples taken from regions of the carcass in contact with the packaging film (rump, mid-back and brisket) and those from areas of the carcass exposed to the gas phase within the body cavity (renal, abdominal-wall and inside hindleg). For example, after 4 and 10 wk storage the B.thermosphacta count on the forelegs and neck was 1.2×10^4 and 8.2×10^6/cm², the mean of the rump, mid-back and brisket sites was 2.5×10^3 and 1.1×10^6/cm², and the mean of the renal, abdominal-wall and inside hindlegs was 10^2 and 3×10^4/cm², respectively.

Off-odor of packaged carcasses

When packs stored for 6–10 wk were opened, stronger off-odors were detected from the N_2-packs than from the CO_2-packs. Hydrogen-sulfide and sour odors were detected in the N_2-packs stored for 6–10 wk. From the CO^2-packs, a slightly sour odor was detected at 8 wk, and hydrogen-sulfide and sour odors at 10 wk. After the carcasses were hung in air at 10°C for 1 hr, these odors dissipated, leaving only a very slightly sour odor from the carcass which had been stored for 10 wk in nitrogen.

Spoilage in air at 5°C

The primal cuts prepared from stored carcasses were stored at 5°C in polyethylene bags and examined for spoilage (off-odor). In Fig. 3, the number of days to spoilage of the six primals is plotted against the number of weeks the packaged carcasses had been stored at 0°C.

Primals removed from packs 1 hr after packaging (zero time) spoiled in 7 days. The longer the carcasses were stored in nitrogen, the shorter the time to spoilage of the aerobically stored primals. Off-odors developed more slowly from primals prepared from
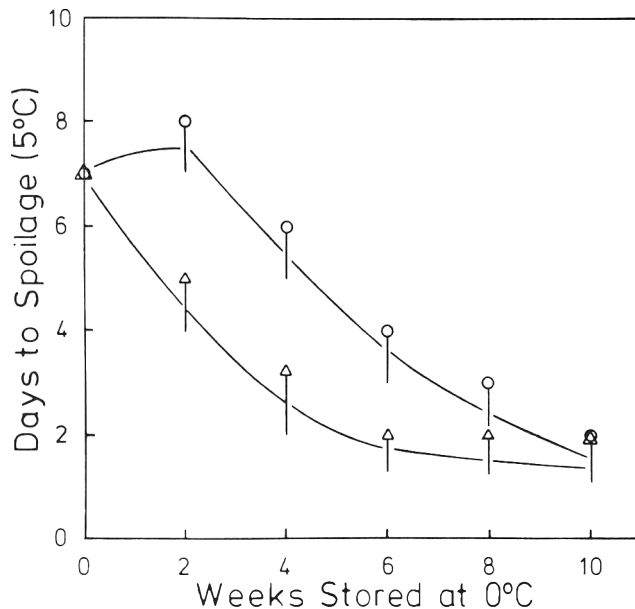
carcasses which had been stored in CO_2-packs. However, after carcasses had been stored for 10 wk, there was essentially no difference in the spoilage time of primals from the two packaging treatments.

When aerobic storage of the loins at 5°C had resulted in spoilage, separate microbiological samples were taken from three sites on the loins. Examination of the microbial counts by analysis of variance showed that the sites differed significantly in their microbial flora. The counts of B.thermosphacta and gram-negative bacteria were, on average, about one log unit higher on the outer, fatty-tissue surface than on the lean end or the inner, connective-tissue surface of the loins. On the other hand, the numbers of lactic-acid bacteria were about 0.6 of a log unit higher on the lean end of the loin than on the other two surfaces sampled. The counts of lactic-acid bacteria, B.thermosphacta, and gram-negative fermentative and oxidative bacteria on the outer, fatty-tissue surface of the loins at spoilage are shown in Table 1. The dominant flora on aerobic spoilage of loins prepared from carcasses 1 hr after packaging (o wk) was B.thermosphacta, pseudomonads, and moraxella-like organisms.

The flora at spoilage on loins from carcasses stored in nitrogen for 2 wk was still dominated by the same types of organisms but with the addition of significant numbers of lactic-acid bacteria. As the storage time in the N_2-packs increased from 4 to 10 wk, gram-negative oxidative bacteria progressively became a smaller proportion of the loin flora at the time of aerobic spoilage. On the other hand, gram-negative fermentative bacteria (principally Enterobacteriaceae with some aeromonads) progressively increased. B.thermosphacta formed a significant proportion of the spoilage flora at all times. Between 6 and 10 wk, lactic-acid bacteria outnumbered the other flora.

On loins from carcasses which had been stored in carbon dioxide for 2 and 4 wk, B.thermosphacta, pseudomonads and moraxella-like organisms dominated the aerobic spoilage flora along with significant numbers of lactic-acid bacteria. When carcasses were stored for 6–10 wk in carbon dioxide, B.thermosphacta and lactic-acid bacteria formed the predominant flora of the loin at spoilage with gram-negative bacteria being only a minor component.

Estimation of micro-flora on plating media

The gram-negative count was frequently significantly lower on VRB agar than that obtained on peptone-salts, or TYSG agars.

Table 1—Log numbers of microorganisms on lamb loins[a] when spoiled in air at 5°C

Carcass gas treatment	Wk carcasses stored at 0°C	Days loins stored at 5°C	Lactic-acid bacteria	B.thermosphacta	Gram-negative[b] fermentative	Gram-negative[c] oxidative
N₂	0	7	< 6.00	7.68	< 5.00	8 08
	2	5	7.60	8.47	< 5.00	8 18
	4	3	6.71	8.04	< 5.00	7 02
	6	2	7.80	7.76	5.15	5.60
	8	2	7.45	6.97	5.93	4 00
	10	2	8.18	6.49	7.02	4 30
CO₂	0	7	< 6.00	7.96	< 5.00	7 70
	2	8	7.00	8.60	< 5.00	7 95
	4	6	7.05	8.36	< 5.00	8 45
	6	4	7.16	8.42	< 5.00	< 5 00
	8	3	7.30	7.54	4.48	4.48
	10	2	7.71	6.23	5.57	4.42

[a] Samples from the fatty tissue on the outer surface of the loin.
[b] Enterobacteriaceae, and aeromonads.
[c] Pseudomonads, alteromonads, moraxella- and acinetobacter-like organisms.

This was mainly due to the failure of moraxella-like bacteria, alteromonads and aeromonads to grow on VRB agar. Since aeromonads were commonly present, the oxidase-positive count on peptone-salts agar was of little value in estimating the aerobic gram-negative flora.

All colonies that were examined from OSGY agar were yeasts. Only rarely were colonies growing on STAA agar not B.thermosphacta. Such rare colonies could be distinguished by their colonial morphology and an oxidase-positive reaction when the plates were flooded with oxidase reagents.

The greatest difficulty encountered was in counting the lactic-acid bacteria. In some samples, particularly those from carcasses stored in carbon dioxide, a higher count of lactic-acid bacteria was given by APT and MRS than by TYSG agar. However, on APT agar, the other flora formed such large colonies that overcrowding frequently occurred. On MRS agar there was a considerable proportion of colonies that were identified as B.thermosphacta or Enterobacteriaceae and which often did not give an unambiguous catalase positive reaction when tested from the MRS plates. A large number of colonies therefore had to be picked from MRS to TYSG or APT to enable the catalase test to be done successfully. When MRS plates were incubated anaerobically, it was often difficult to sub-culture small colonies (ca 1 mm diameter). Many such colonies were identified as colonies of B.thermosphacta in which it appeared most of the cells were non-viable. In spite of these problems, MRS was the only medium on which lactic-acid bacteria could be identified when the other flora considerably outnumbered these organisms.

DISCUSSION

THIS STUDY has shown that, when lamb carcasses are packaged, a carbon dioxide atmosphere is more efficient than nitrogen flushing in delaying the growth of gram-negative oxidative and fermentative bacteria, B.thermosphacta, and lactic-acid bacteria. This agrees with the data of Sander and Soo (1978), Enfors et al. (1979), Hess et al. (1980), Blickstad et al. (1981), and Erichsen and Molin (1981) for meat derived from other animal species. As a consequence the development of off-odors is delayed both while the lamb carcasses are still packaged and when primal cuts prepared from the carcasses are subsequently stored aerobically at 5°C. However, after the lamb carcasses were stored for 8–10 wk at 0°C, the difference between nitrogen and carbon dioxide treatments in the extent of off-odor formation became considerably less. One reason for this may be the significant decrease in the carbon dioxide concentration in the gas phase within the packs during storage.

Of interest is the very high count of microorganisms in the purge fluid from the packaged carcasses. It is therefore difficult to know whether the production of sour or hydrogen sulfide odors in the packaged carcasses is a consequence of microbial growth in the purge fluid or of microbial growth on the predominantly fatty and

connective tissue surfaces of the carcasses. Microbial counts of the carcass tissue alone could give a false impression of the role some organisms could play in spoilage. For example, the mean count of gram-negative bacteria on the carcasses stored in nitrogen was not greater than $10^5/cm^2$, but the count of these bacteria in the purge was about $10^8/mL$.

The high pH of the purge fluid suggests that microbial growth in it will resemble microbial growth on high pH (pH $\geq$ 6) lean. Indeed the detection of alteromonads in the purge in significant numbers is in agreement with this. Furthermore it has been shown (Egan and Grau, 1981; Grau, 1981; 1983) that growth of B.thermosphacta and of Enterobacteriaceae occurs more readily on fatty tissue than on lean of low pH (pH $\leq$ 5.7). Therefore, both purge fluid and most of the carcass surface provide ecological environments in which a more mixed flora is able to grow than could be expected to grow on low pH lean. This can be seen in the data for carcasses stored in nitrogen where both B.thermosphacta and Enterobacteriaceae form a significant proportion of the population both in the purge fluid and on the carcass surfaces. Patterson and Gibbs (1978) and Shaw et al. (1980) also noted that B.thermosphacta formed a large proportion of the total flora on vacuum-packed primal cuts of lamb.

There appears to be little evidence in the microbiological results obtained here to suggest that lactic-acid bacteria inhibited the growth of the other organisms. For example, in the purge from carcasses stored in carbon dioxide for 6–10 wk, the count of lactic-acid bacteria was above $10^8/mL$ yet the count of both B.thermosphacta and gram-negative bacteria increased considerably during this period.

The aerobic spoilage flora of loins from carcasses that had not been stored in nitrogen or carbon dioxide consisted of moraxella-like bacteria, pseudomonads and B.thermosphacta. B.thermosphacta and moraxella-like bacteria were also found to dominate the aerobic flora of spoiled lamb loins by Shaw et al. (1980). These authors also observed that the aerobic spoilage flora of loins, which had previously been stored in vacuum-packs, was still dominated by B.thermosphacta and gram-negative oxidative bacteria. In the experiments reported here, however, the gram-negative oxidative bacteria became progressively a smaller proportion of the aerobic spoilage flora of loins as the storage time of the carcasses increased.

REFERENCES

Blickstad, E., Enfors, S.-O., and Molin, G. 1981. Effect of hyperbaric carbon dioxide pressure on the microbial flora of pork stored at 4 or 14°C. J. Appl. Bacteriol. 50: 493.

Bruck, J.D. 1982. Nonstaining (KOH) method for determination of gram reactions of marine bacteria. Appl. Environ. Microbiol. 44: 992.

Collins, C.H. and Taylor, C.E.D. 1967. "Microbiological Methods", 2nd ed., p.138. Plenum Press, New York.

Edwards, P.R. and Ewing, W.H. 1972. "Identification of Enterobacteriaceae," 3rd ed., p.347. Burgess Publishing Co., Minneapolis, MN.

Egan, A.F. and Grau, F.H. 1981. Environmental conditions and the role of Brochothrix thermosphacta in the spoilage of fresh and processed meat. In "Psychrotrophic Microorganisms in Spoilage and Pathogenicity," (Ed.)

Relationships of Hydrophobicity and Net Charge to the Solubility of Milk and Soy Proteins

SHIGERU HAYAKAWA and SHURYO NAKAI

------------ ABSTRACT ------------

Protein hydrophobicity of 42 native and partially denatured milk and soy protein samples was determined fluorometrically by using three probes, 1-anilino-8-naphthalencsulfonate (ANS), cis-parinarate (CPA) and 1,6-diphenyl hexatriene (DPH), and chromatographically by using Phenyl Sepharose CL-4B (PSC). PSC and ANS hydrophobicities correlated well to the protein insolubility determined at zero zeta potential, whereas no significant correlation was observed between CPA hydrophobicity and protein insolubility. When backwards stepwise regression analysis was applied to 189 data of protein insolubility, a significant correlation ($P < 0.001$) was obtained between PSC hydrophobicity, zeta potential and protein insolubility. It is suggested that the aromatic hydrophobicity, in conjunction with zeta potential, may play a more important role in protein solublity than the aliphatic hydrophobicity.

INTRODUCTION

SOLUBILITY OF PROTEINS has been thought to be the most important factor for their functionality (Kinsella, 1976). In the extraction and precipitation of proteins during production, information about changes in solubility is of prime importance (Hermansson, 1973). Good solubility can markedly expand potential applications of proteins. Heat treatment causes the loss of functional properties and its effect is usually measured as a loss of solubility (Wolf, 1970).

Protein solubility may affect other functional properties. Although some authors reported that emulsifying properties and solubility were not well correlated (Aoki et al., 1980, McWatters and Holmes, 1979) better correlations to the various protein functionalities were obtained when hydrophobic parameters were used in conjunction with solublity (Voutsinas et al., 1983a, b; Voutsinas and Nakai, 1983; Townsend and Nakai, 1983). Li-Chan et al. (1984) also reported that solubility parameters were influential in emulsifying properties for samples with low (<50%) solubility.

Solubility of proteins has been studied for a long time. However, reliable quantitative relationships between solubility and structures of proteins have not been demonstrated. Measurements of the solubility of amino acids and related substances have been used to assess the contribution of the side chains to the free energy of transfer which was used for calculation of hydrophobicity (Nozaki and Tanford, 1971). McGowan and Mellors (1979) reported the relationships between the solubility of amino acids and hydrophobicity.

Bigelow (1967) has suggested that charge frequency and hydrophobicity are likely to be the two structural features which have the greatest influence on the solubility. Melander and Horvath (1977) determined the salt effect on protein solubility and suggested that it could be described by the two antagonistic effects on electrostatic and hydrophobic interactions.

The purpose of the present study was to determine quantitatively the effect of hydrophobicity and charge on the solubility of milk and soy proteins.

MATERIALS & METHODS

Materials

α_{s1}-Casein was prepared by the DEAE-cellulose chromatographic method of Thompson (1966). κ-Casein was prepared by the method of Zittle and Custer (1963). Soy 7S and 11S globulins were prepared by the method of Thanh and Shibasaki (1976) from defatted soy flour (Canasoy Ent. Ltd., Vancouver, BC). α-Casein (C 7891), α-lactalbumin (L 6010), β-lactoglobulin (L 0130), bovine serum albumin (A 6003), ovalbumin (A 2512) and soybean trypsin inhibitor (T 9003) were purchased from Sigma Chemicals (St. Louis, MO). β-Casein was purchased from Chemical Dynamics Corp. (South Plainfield, NJ). Phenyl Sepharose CL-4B (17-0810-01, Lot. GL-19419) was the product of Pharmacia Fine Chemicals (Uppsala, Sweden). Magnesium salt of 1-anilino-8-naphthalenesulfonic acid was prepared from technical grade sodium salt (Eastman Kodak Co., Rochester, NY) according to the method of Weber and Young (1964). cis-Parinaric acid was purchased from Molecular Probes (Junction City, OR). 1,6-Diphenyl hexatriene and 3,3'-dimethylbiphenyl were obtained from Aldrich Chemical Co. (Milwaukee, WI).

Protein insolubility

The solutions of 42 native and denatured milk and soy protein samples were prepared as follows. (a) Native proteins were dissolved in 0.01M phosphate buffer (pH 7) at the protein concentration of 0.1 or 0.2 %. (b) Native protein solutions were heated at different temperatures for 10 min in the presence or absence of 10 mM mercaptoethanol and then cooled in ice-water. (c) Native protein solutions were adjusted to pH 2 with 1N HCl, heated at 95°C for 10 min, cooled in ice-water and then readjusted to pH 7 with 1N NaOH. (d) Native protein solutions were adjusted to pH 12 with 1N NaOH, incubated for 2 hr at room temperature and readjusted to pH 7 with 1N HCl. (e) Native proteins were dissolved in 0.086 M Tris-0.09M glycine-0.004 M EDTA (pH 8) buffer containing 8M urea and 100 mM mercaptoethanol and then after standing for 2 hr at room temperature urea was removed by a Sephadex G-25 gel filtration column equilibrated with 0.01M phosphate buffer (pH 7). All of the above solutions of protein samples were centrifuged at 6,000 × g following the adjustment to the specified pH and holding for 2 hr at room temperature. Protein content in the supernatant was measured by the method of Lowry et al. (1951) or Bio Rad protein assay method (Richmond, CA). In this work, protein insolubility was expressed as a percentage of insoluble protein in the total protein in suspension at the specified pH.

Hydrophobicity determination

Hydrophobicity measurement of the protein samples using hydrophobic chromatography was performed on a Phenyl Sepharose CL-4B column (1.5 × 4.5 cm) equilibrated with 0.01M phosphate buffer (pH 7). Protein was eluted with 0.01M phosphate buffer (pH 7) with an exponential gradient of isopropanol, 0 - 50%. Isopropanol may be more suitable than acetonitrile or methanol as an organic modifier in hydrophobic chromatography for hydrophobic peptides and proteins because proteins are more soluble in this alcohol, and moreover the concentration for elution may be decreased due to its high elution efficiency (Mahoney and Hermodson, 1980; Wilson et al., 1981; Heukeshoven and Dernick, 1982). All of the proteins were soluble in 50% isopropanol. Hydrophobicity was expressed as weight average center point of protein peak monitored by measuring absorbance at 280 nm. The elution volume was expressed as the corresponding per cent isopropanol concentration.

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Hydrophobicity was also determined using hydrophobic fluorescence probes, 1-anilino-8-naphthalenesulfonate (ANS), cis-parinarate (CPA) and 1,6-diphenyl-hexatriene (DPH). Measurements were performed according to the method of Kato and Nakai (1980) in the absence of SDS. Each protein sample (2 mL) was serially diluted with 0.01M phosphate buffer (pH 7) to obtain protein concentrations ranging from 0.008 – 0.03%. Then, 10 μL ANS (8.0 mM in 0.1M phosphate buffer, pH 7), CPA (3.6 mM in absolute ethanol containing equimolar butylated hydroxytoluene) or DPH (1.0 mM in tetrahydrofuran) solution was added. Fluorescence intensity (FI) was measured with an Aminco Bowman spectrophotofluorometer, No. 4-8202 at wavelengths (λ_{ex}, λ_{em}) of (390, 470), (325, 420) and (360, 430) for ANS, CPA and DPH, respectively. The FI reading was standardized by adjusting the reading of the fluorometer to 30% full scale for ANS in methanol, 70% full scale for CPA in decane and 60% full scale for DPH in decane. The net FI at each protein concentration was determined by subtracting FI of each solution without probe from that with probe. The initial slope (S_0) of the FI versus protein concentration (%) plot, that was calculated by linear regression analysis with a Monroe 1880 programmable calculator, was used as an index of the protein hydrophobicity.

Net charge determination

Net charge was measured with a particle microelectrophoresis apparatus (Pen Kem, Laser Zee Model 501, Bedford Hills, NY). Protein particle suspension was prepared by homogenizing the mixture of 0.1% protein solution and 3,3'-dimethylene biphenyl (100:3) using a Brinkmann Polytron at 3000 rpm for 30 sec and then diluted with 40 fold-volume of 0.01M acetate buffer with pH ranging from 4 – 6. Buffer with a constant specific conductance (0.8 m mhos/cm) was used because specific conductance, which depends on salt concentration, strongly affected the measurement of the electrophoretic mobility. The electrophoretic mobility is given by

$$\mu = V/E \qquad (1)$$

where V is the particle velocity and E is the applied field strength. Mobilities were measured at an applied potential difference of 150 V. The following equation was used to calculate the zeta potential (Kruyt, 1952)

$$\mu = \zeta\epsilon/4\ \pi\eta \qquad (2)$$

where ζ is the zeta potential, ϵ is the dielectric constant and η is the viscosity of the suspension medium. Absolute value of zeta potential in mV derived from mobility measurement was used in the following computations.

Statistical analysis

Simple linear regression analysis was performed with a Monroe 1880 programmable calculator. Backward stepwise multiple regression analysis was carried out with the UBC Triangular Regression Package, while 3-dimensional surface plots were generated by using the UBC Surface Visualization Routines program, with an Amdahl 470 V/8 computer.

RESULTS & DISCUSSION

Correlation of hydrophobicity to protein solubility

Although hydrophobicity determined by using CPA was well correlated to the protein functionalities (Kato and Nakai, 1980; Nakai et al., 1980; Townsend and Nakai, 1983; Voutsinas et al., 1983a, b; Kato et al., 1983; Li-Chan et al., 1984), the CPA hydrophobicity was influenced by a small change of pH. This pH effect was also observed by Kato et al. (1984). Hydrophobicities for most of the proteins determined by using hydrophobic chromatography as well as dissociable fluorescence probes are affected by pH. Therefore, hydrophobicity was determined at a constant pH (7.0) to eliminate the pH effect. Fig. 1. 2 and 3 show the correlation between CPA, ANS or PSC hydrophobicity and protein insolubility of 42 protein samples measured at the zeta potential of zero mV. Insolubility 'at zero zeta potential' was used to remove the charge effects from the insolubility data. CPA hydrophobicity, as well as DPH hydrophobicity (result not shown), did not

show significant correlation to the insolubility (Fig. 1). Conversely, ANS hydrophobicity showed a good correlation to the insolubility (Fig. 2) with correlation coefficient 0.592 (P < 0.001). In this correlation computation, the data of native bovine serum albumin was eliminated because this protein was capable of combining with a large variety of anions including such simple ions as chloride (Scatchard et al., 1950; Laurence, 1952) and some of the binding sites did not bind alkyl carboxylates at all, whereas both alkyl sulfates and sulfonates were strongly bound (Reynolds et al., 1967, 1968). There was a more highly significant correlation (r = 0.775, P < 0.001) between the PSC hydrophobicity and the insolubility (Fig. 3) with the regression equation:

$$[\%\text{ insoluble protein}] = 9.30 + 2.93\ PSC \qquad (3)$$

Although the theoretical basis of binding of protein to phenyl Sepharose is not clear, the elution position depends on the hydrophobicity alone when the proteins are similar in structure (Burley and Sleigh, 1983) and proteins with many aromatic amino acids on the surface may bind well to phenyl Sepharose through π-π bond interaction.

Fig. 4 and 5 show the effect of heating on the relative hydrophobicities and the insolubility of soybean trypsin inhibitor and β-lactoglobulin. Insolubility was measured
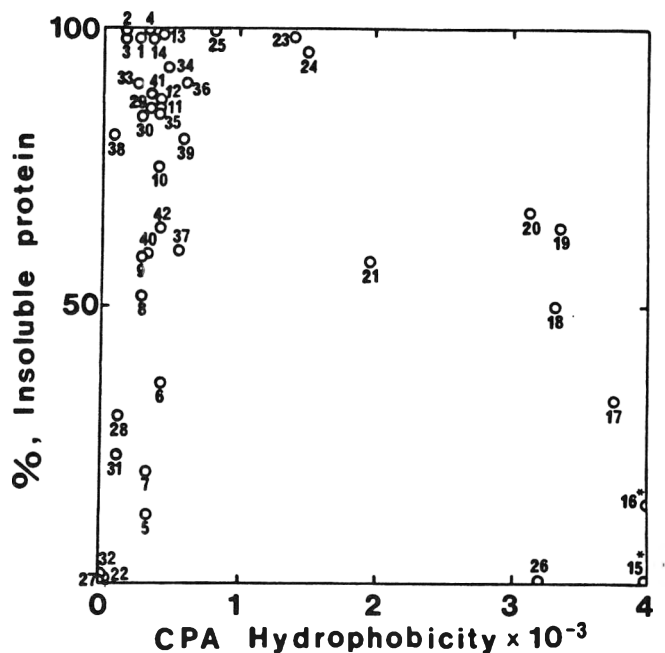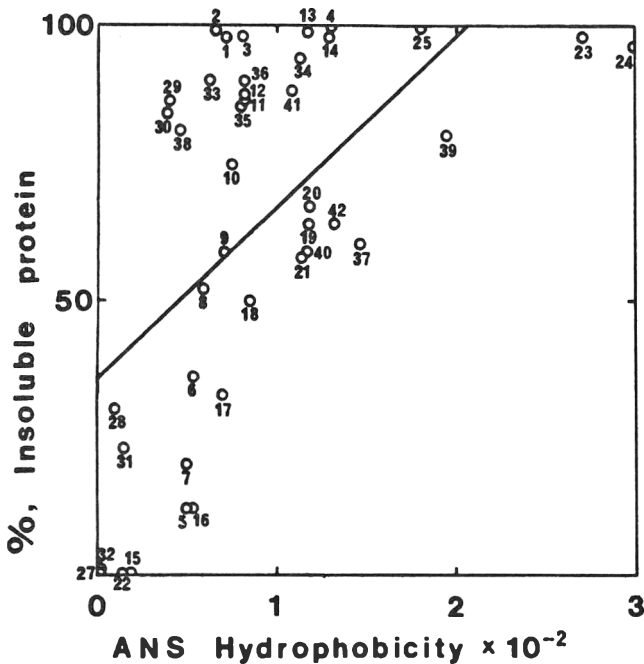

Fig. 1–Relationship between protein insolubility at zero zeta potential and hydrophobicity measured fluorometrically using cis-parinaric acid (CPA). (1) α-casein; (2) α_{sl}-casein; (3) β-casein; (4) κ-casein; (5) native α-lactalbumin (α-La); (6) α-La heated at 90°C; (7) – (13) α-La heated at 30, 40, 45, 50, 60, 80 and 90°C in the presence of 10 mM mercaptoethanol (ME); (14) α-La treated with 8M urea and 100 mM ME; (15) native β-lactoglobulin (β-Lg); (16) – (20) β-Lg heated at 75, 80, 85, 90 and 95°C; (21) β-Lg treated with urea and ME; (22) native ovalbumin; (23) ovalbumin heated at 85°C; (24) bovine serum albumin (BSA) treated with urea and ME; (25) BSA heated at 90°C; (26) native BSA; (27) native soybean trypsin inhibitor (STI); (28) STI heated at 95°C; (29) STI heated at 95°C in the presence of ME; (30) STI treated with urea and ME; (31) STI heated at 95°C at pH 2; (32 STI treated with alkali (pH 12); (33) native soy 7S globulin (7S); (34) 7S heated at 95°C; (35) 7S treated with urea and ME; (36) 7S heated at 95°C at pH 2; (37) 7S treated with alkali; (38) native soy 11S globulin (11S); (39) 11S heated at 95°C; (40) 11S treated with urea and ME; (41) 11S heated at 95°C at pH 2; (42) 11S treated with alkali. (*Hydrophobicity was higher than 4000.)

'at zero zeta potential'. There are two different types of proteins among the heat sensitive proteins. For the majority of proteins tested all of CPA, ANS and PSC hydrophobicities and the insolubility are positively correlated as temperature changes (Fig. 4). However, for β-lactoglobulin, as well as bovine serum albumin, CPA hydrophobicity, unlike others, is negatively correlated as temperature changes (Fig. 5). It is well known that native bovine serum albumin and

β-lactoglobulin show strong interaction with aliphatic hydrocarbon chains (Wishnia and Pinder, 1966; Shanbhag and Axelsson, 1975; Chen and Morawetz, 1981; Mattarella and Richardson, 1983), triglyceride (Smith et al., 1983) and fatty acid (Kato and Nakai, 1980; Kato et al., 1983; Voutsinas et al., 1983a) in aqueous environment. Decrease in affinity of these proteins with aliphatic hydrocarbon chains by heat denaturation has been observed (Kato et al., 1983; Voutsinas et al., 1983a). cis-Parinaric acid is com-

Fig. 2—Relationship between protein insolubility at zero zeta potential and hydrophobicity measured fluorometrically using 1-anilino-8-naphthalenesulfonate (ANS). Sample identification is the same as in Fig. 1. Regression equation: [% insoluble protein] = 36.2 + 0.309 ANS (r = 0.592, P < 0.001).
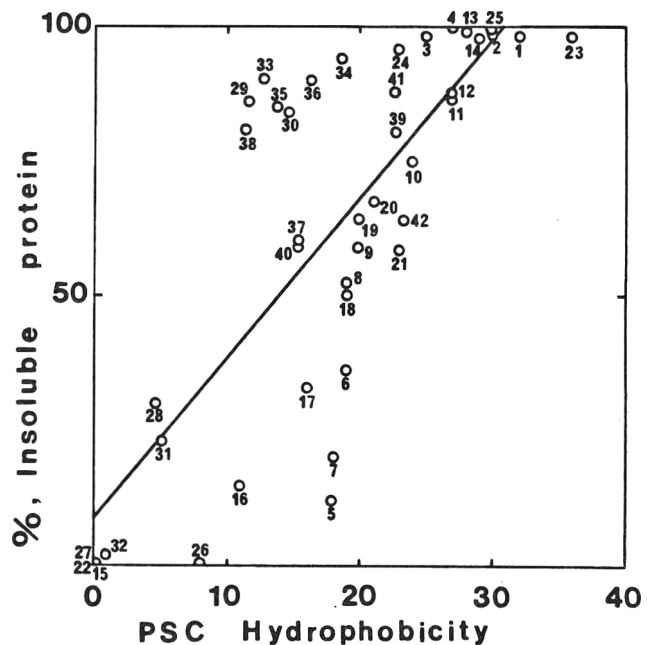

Fig. 3—Relationship between protein insolubility at zero zeta potential and hydrophobicity measured by the hydrophobic chromatography on a Phenyl Sepharose CL-4B column. Sample identification is the same as in Fig. 1. Regression equation: [% insoluble protein] = 9.30 + 2.93 PSC (r = 0.775, P < 0.001).
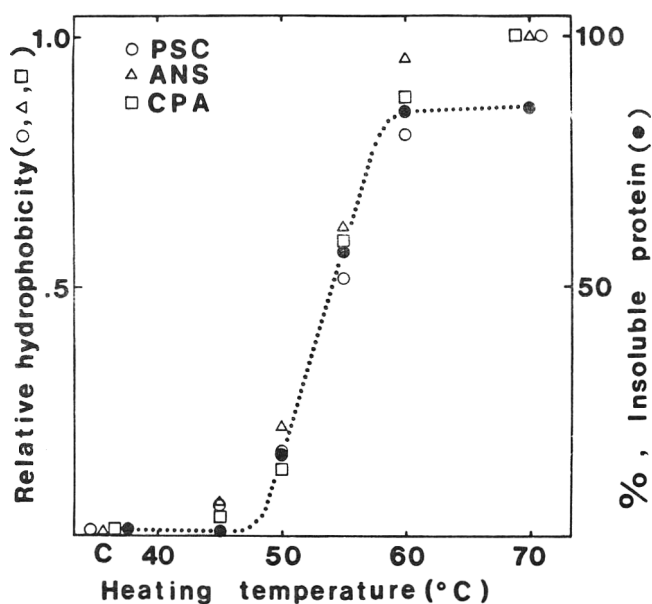

Fig. 4—Effect of heating on protein insolubility at zero zeta potential and hydrophobicity of soybean trypsin inhibitor. Protein solution (0.2%) was heated at pH 7 for 10 min in the presence of 10 mM mercaptoethanol. Relative hydrophobicity represents the value against that of protein heated at 70°C.
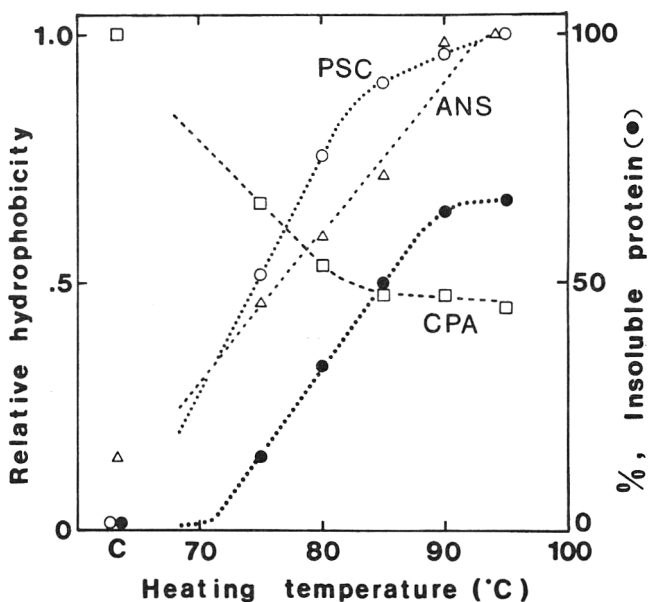

Fig. 5—Effect of heating on protein insolubility at zero zeta potential and hydrophobicity of β-lactoglobulin. Relative hydrophobicity for ANS and PSC represents the value against that of protein heated at 95°C and relative hydrophobicity for CPA represents the value against that of unheated protein.

posed of an aliphatic hydrocarbon chain, while anilino-naphthalenesulfonate is composed of aromatic rings; aromatic groups are also attached to agarose in phenyl Sepharose. Therefore, it is postulated that the binding sites of CPA on protein molecules may differ from those of ANS and phenyl Sepharose. Aromatic solvent may possess different characteristics from aliphatic solvent in terms of polarity. Snyder (1978) has suggested that the polarity scale is essential for classifying the solvents and aromatic solvents are classified into a different group from aliphatic hydrocarbons. Kamlet et al. (1977) found that although a plot of π^* scale of solvent polarities against ν_{max} (maximum reaction rate) values of non-hydrogen-bonding solvents showed considerable scatter, the correlation significantly improved when either aliphatic solvents or aromatic solvents was taken alone. Specific retardation of aromatic amino acids on crosslinked dextran gel was also observed (Porath, 1960). Hofstee and Otillio (1978) found that some proteins were preferentially bound by the aliphatic adsorbent, others by the aromatic adsorbent using agaroses substituted by n-alkyl-ligands with and without terminal phenyl group. Therefore, it is reasonable to classify the hydrophobicity of protein into the aliphatic hydrophobicity due to aliphatic amino acid residues and the aromatic hydrophobicity due to aromatic amino acid residues. CPA seems to be useful for determining the aliphatic hydrophobicity, and ANS and phenyl Sepharose for determining the aromatic hydrophobicity.

Good correlations of ANS and PSC hydrophobicities to the protein insolubility (Fig. 2 and 3) indicate that the exposed aromatic amino acids may play an important role in the protein insolubility. Although the theoretical basis of importance of aromatic amino acid residues in the protein solubility is unknown, the important role of aromatic amino acid residues may partly owe to high hydrophobicity of aromatic amino acids calculated from free energy of transfer (Nozaki and Tanford) and from hydrophobic fragmental constant (Rekker, 1977). Although hydrophobicity of aromatic amino acids is higher than that of aliphatic amino acids, the hydrophobicity of aromatic amino acids may be underestimated because its π electrons lead to stronger van der Waals attraction to water molecules. The standard free energy of transfer of ligand molecules to hydrophobic regions on protein molecules may be more negative than that for a hydrocarbon because the process of binding may involve elimination of a hydrocarbon-water

interface at protein surface (Tanford, 1973). Therefore, hydrophobic interaction between aromatic amino acid residues may be much stronger when the elimination of water in the process of aggregation takes place.

Correlation of zeta potential and hydrophobicity to the protein solubility

Another important factor affecting solubility is net charge on the protein molecules. Net charge effect is usually shown as pH effect, that is, protein solubility is displayed as a function of pH (Wolf, 1970). However, proteins have different isoelectric points that can be shifted by denaturation and chemical modification such as acetylation and succinylation. It is quite difficult to compare the charge effect, as a function of pH, on the functional properties of proteins having different isoelectric points. It is useful to use zeta potential instead of pH as a variable. The aggregate size of soy protein particles was correlated well to zeta potential (Chan et al., 1982). Protein insolubility was affected by zeta potential and proteins showed the maximum insolubility at zeta potential near zero (Fig. 6).

Table 1 shows the results of backward stepwise multiple regression analysis which is applied to protein insolubility measured 'at different zeta potentials' ranging from 0 – 30 mV as functions of hydrophobicity and the absolute values of zeta potential. There was a good correlation (P < 0.001) when ANS hydrophobicity was used as an independent variable representing hydrophobicity. The data of native bovine serum albumin were again eliminated from analysis. A much better correlation (P < 0.001) was obtained when PSC hydrophobicity was used. Insolubility significantly (P < 0.001) correlated with PSC hydrophobicity, negative value of square of zeta potential and negative value of the interaction of zeta potential (ZP) and PSC hydrophobicity:

$$[\% \text{ insoluble protein}] = 3.43 \, PSC - 0.057 \, PSC \cdot ZP - 0.042 ZP^2 + 4.4 \qquad (4)$$

Meanwhile, the regression equation obtained using CPA data as an independent variable showed that CPA hydrophobicity correlated negatively to the insolubility which was theoretically unagreeable even though it was significant. Therefore, it is suggested that the aromatic hydrophobicity as well as zeta potential are responsible for the protein solubility. Surface hydrophobicity determined by using CPA
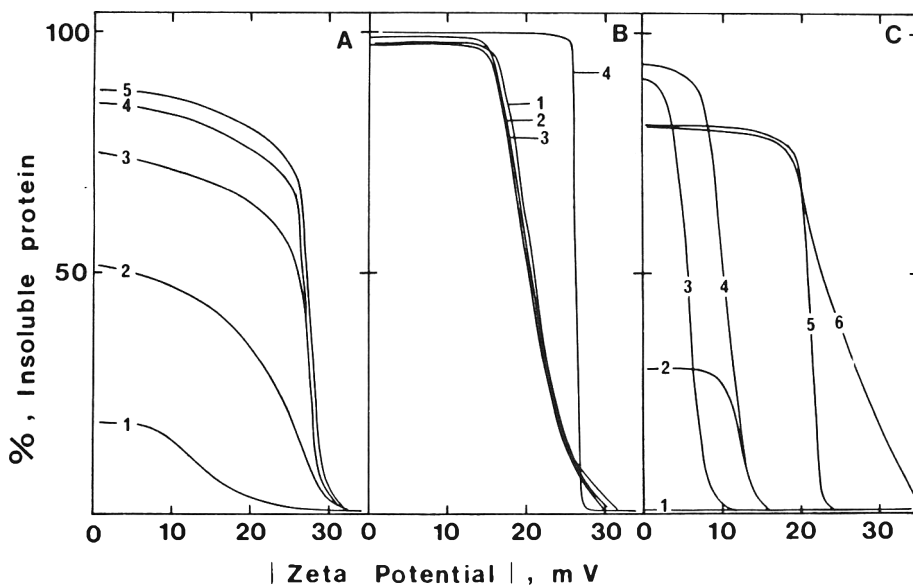

Fig. 6—Effect of zeta potential on protein insolubility of several proteins. [A] α-lactalbumin; (1) unheated, (2) heated at 40°C, (3) 50°C, (4) 60°C, (5) 80°C in the presence of 10 mM mercaptoethanol. [B] Caseins; (1) α-casein, (2) α_{s1}-casein, (3) β-casein, (4) k-casein. [C] Soy proteins; (1) unheated and (2) heated soybean trypsin inhibitor, (3) unheated and (4) heated soy 7S globulin, (5) unheated and (6) heated soy 11S globulin.

and the protein solubility. Surface hydrophobicity determined by using CPA and the protein solubility were well correlated to some functional properties (Voutsinas et al., 1983a, b; Voutsinas and Nakai, 1983; Townsend and Nakai, 1983; Li-Chan et al., 1984). This indicates that a combination of the aliphatic and aromatic hydrophobicities may be important to explain the protein functionalities.

Three dimensional surface plots of the insolubility of milk and soy proteins

Three dimensional surface plots were generated by a computer to help visualization of the relationship between zeta potential, PSC hydrophobicity and the protein insolubility 'at different zeta potentials' for all of the poroteins determined (Fig. 7) and those except soy proteins (Fig. 8). These figures clearly indicated that the protein insolubility increases with an increase of hydrophobicity and with a decrease of zeta potential. A much smoother figure was obtained after eliminating the data of soy proteins. This means that the insolubility property of milk proteins is slightly different from that of soy proteins and suggests that there are some other factors which may affect protein solubility such as structural effects, especially the difference in conjugation (e.g. phosphoproteins vs. glycoproteins).

In conclusion, protein hydrophobicity may be subclassified into the aliphatic hydrophobicity and the aromatic hydrophobicity and the contribution of each hydrophobicity to protein functionality is desirable to be assessed separately. Backward stepwise multiple regression analysis showed that the aromatic hydrophobicity and zeta potential were major factors for the protein solubility.

Table 1—Multiple regression models for prediction of the protein insolubility

Dependent variable	Variable description	Regression coefficient	F-ratio	F-probability	β-value
% Insoluble protein (PSC)[a] ($n = 189$; $R^2 = 0.612$, $P < 0.001$; S.E.[e] $= 23.2$)	Constant	4.44	0.72	0.396	0.120
	PSC	3.43	94.70	0.000	0.841
	ZP^2	-0.0416	7.67	0.006	-0.295
	PSC x ZP	-0.0573	7.04	0.009	-0.366
% Insoluble protein (ANS)[b] ($n = 183$; $R^2 = 0.480$, $P < 0.001$; S.E. $= 26.9$)	Constant	13.4	5.15	0.024	0.362
	ANS	0.781	62.34	0.000	1.404
	ANS^2	-0.0014	22.51	0.000	-0.761
	ZP^2	-0.042	10.18	0.002	-0.302
	ANS x ZP	-0.010	8.27	0.005	-0.387
% Insoluble protein (CPA)[c] ($n = 189$; $R^2 = 0.258$, $P < 0.001$; S.E. $= 32.0$)	Constant	70.9	221.9	0.000	1.915
	CPA^2	-0.0000011	14.61	0.000	-0.242
	ZP	-1.89	47.40	0.000	-0.435
% Insoluble protein (DPH)[d] ($n = 125$; $R^2 = 0.275$, $P < 0.001$; S.E. $= 32.6$)	Constant	71.1	150.4	0.000	1.878
	DPH	-0.180	7.61	0.007	-0.554
	DPH^2	0.0004	7.75	0.006	0.559
	ZP^2	-0.0635	37.67	0.000	-0.476

[a,b,c,d] Insolubility correlated to PSC, ANS, CPA and DPH hydrophobicity, respectively.
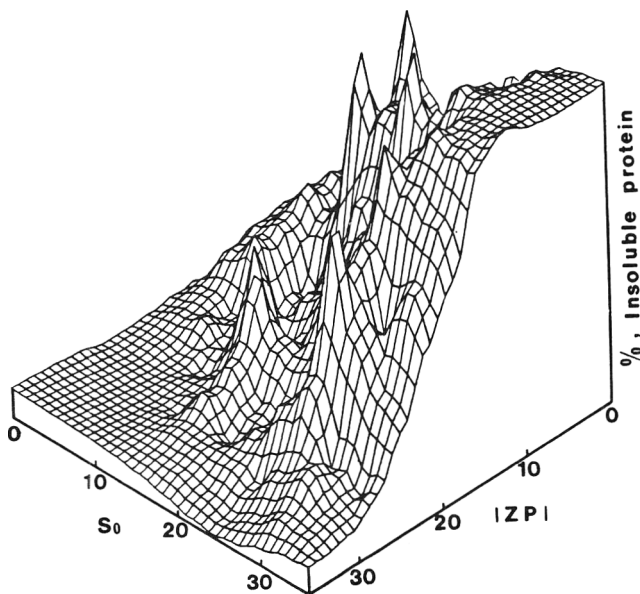[e] Standard error of estimate.

Fig. 7—Three dimensional surface plot of protein insolubility as functions of zeta potential (ZP) and PSC hydrophobicity (S₀). Protein insolubility was measured at different zeta potentials ranging from 0 – 30 mV.
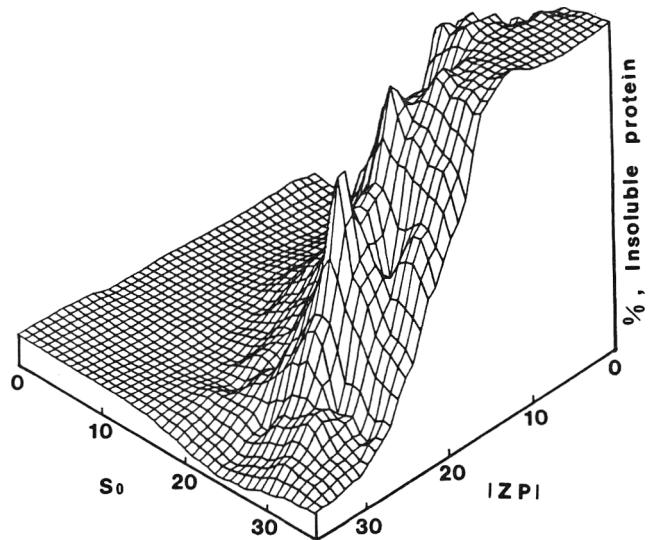
Fig. 8—Three dimensional surface plot of protein insolubility exclusing the data of soy proteins as functions of zeta potential (ZP) and PSC hydrophobicity (S₀).

REFERENCES

Aoki, H., Taneyama, O., and Inami, M. 1980. Emulsifying properties of soy protein. Characteristics of 7S and 11S proteins. J. Food Sci. 45: 534.

Bigelow, C.C. 1967. On the average hydrophobicity of proteins and the relation between it and protein structure. J. Theoret. Biol. 16: 187.

Burley, R.W. and Sleigh, R.W. 1983. Hydrophobic chromatography of proteins in urea solutions. The separation of apoproteins from a lipoprotein of avian egg yolk. Biochem. J. 209: 143.

Chan, M.Y.Y., Bell, D.J., and Dunnill, P. 1982. The relationship between the zeta potential and the size of soya protein acid precipitate particles. Biotechnol. Bioeng. 24: 1897.

Chen, J-L. and Morawetz, H. 1981. Affinity electrophoresis in gels containing hydrophobic substituents. J. Biol. Chem. 256: 9221.

Hermansson, A-M. 1973. Functional properties of proteins for food stability. AULs halvarsskrift 2.

Heukeshoven, J. and Dernick, R. 1982. Reversed-phase high performance liquid chromatography of virus proteins and other large hydrophogic proteins in formic acid containing solvent. J. Chromatogr. 252: 241.

Hofstee, B.H.J. and Otillio, F.N. 1978. Modifying factors in hydrophobic protein binding by substituted agaroses. J. Chromatogr. 161: 153.

Kamlet, M.J., Abboud, J.L., and Taft, R.W. 1977. The solvatochromic comparison method. 6. The π^* scale of solvent polarities. J. Amer. Chem. Soc. 99: 6027.

Kato, A. and Nakai, S. 1980. Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. Biochim. Biophys. Acta 624: 13.

Kato, A., Osako, Y., Matsudomi, N., and Kobayashi, K. 1983. Changes in the emulsifying and foaming properties of proteins during heat denaturation. Agric. Biol. Chem. 47: 33.

Kato, A., Matsuda, T., Matsudomi, N., and Kobayashi, K. 1984. Determination of protein hydrophobicity using a sodium dodecyl sulfate binding method. J. Agric. Food Chem. 32: 284.

Kinsella, J.E. 1976. Functional properties of proteins in food: A survey. Crit. Rev. Food Sci. Nutr. 7: 219.

Kruyt, H.R. 1952. "Colloid Science," Vol. 1. Elsevier, New York.

Laurence, D.J.R. 1952. A study of the adsorption of dyes on bovine serum albumin by the method of polarization of fluorescence. Biochem. J. 51: 168.

Li-Chan, E., Nakai, S., and Wood, D.F. 1984. Hydrophobicity and solubility of meat proteins and their relationship to emulsifying properties. J. Food Sci. 49: 345.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the folin-phenol reagent. J. Biol. Chem. 193: 265.

Mahoney, W.C. and Hermodson, M.A. 1980. Separation of large denatured peptides by reverse phase high performance liquid chromatography. Trifluoroacetic acid as a peptide solvent. J. Biol. Chem. 255: 11199.

Mattarella, N.L. and Richardson, T. 1983. Physicochemical and functional properties of positively charged derivatives of bovine β-lactoglobulin. J. Agric. Food Chem. 31: 972.

McGowen, J.C. and Mellors, A. 1979. Relationships between the solubility of amino acids. J. Appl. Biochem. 1: 423.

McWatters, K.H. and Holmes, M.R. 1979. Influence of pH and salt concentration on nitrogen solubility and emulsification properties of soy flour. J. Food Sci. 44: 770.

Melander, W. and Horvath, C. 1977. Salt effect on hydrophobic interaction in precipitation and chromatography of proteins: An interpretation of the lyotropic series. Arch. Biochem. Biophys. 183: 200.

Nakai, S., Ho, L., Helbig, N., Kato, A., and Tung, M.A. 1980. Relationship between hydrophobicity and emulsifying proerties of some plant proteins. Can. Inst. Food Sci. Technol. J. 13: 23.

Nozaki, T. and Tanford, C. 1971. The solubility of amino acids and two glycine peptides in aqueous ethanol and dioxane solutions. J. Biol. Chem. 246: 2211.

Porath, J. 1960. Gel filtration of proteins, peptides and amino acids. Biochim. Biophys. Acta 39: 193.

Rekker, R.F. 1977. "The Hydrophobic Fragmental Constant," Elsevier, Amsterdam.

Reynold, J.A., Herbert, S., Polet, H., and Steinhardt, J. 1967. The binding of diverse detergent anions to bovine serum albumin. Biochemistry 6: 937.

Reynold, J.A., Herbert, S., and Steinhardt, J. 1968. The binding of some long-chain fatty acid anions and alcohols by bovine serum albumin. Biochemistry 7: 1357.

Scatchard, G., Scheinberg, H.I., and Armstrong, H.S. 1950. Physical chemistry of protein solutions. IV. The combination of human serum albumin with chloride ion. J. Amer. Chem. Soc. 72: 535.

Shanbhag, V.P. and Axelsson, C.G. 1975. Hydrophobic interaction determined by partition in aqueous two-phase system. Partition of proteins in systems containing fatty acid esters of poly(ethylene glycol). Eur. J. Biochem. 60: 17.

Smith, L.M., Fantozzi, P., and Creveling, R.K. 1983. Study of triglyceride-protein interaction using a microemulsion-filtration method. J. Amer. Oil Chem. Soc. 60: 960.

Snyder, L.R. 1978. Classification of the solvent properties of common liquids. J. Chromatg. Sci. 16: 223.

Tanford, C. 1973. "The Hydrophobic Effect: Formaton of Micelles and Biological Membranes," Wiley-Interscience, New York.

Thanh, V.H. and Shibasaki, K. 1976. Major proteins of soybean seeds. A straightforward fractionation and their characterization. J. Agric. Food Chem. 24: 1117.

Thompson, M.P. 1966. DEAE-Cellulose-urea chromatography of casein in the presence of 2-mercaptoethanol. J. Dairy Sci. 49: 792.

Townsend, A-A. and Nakai, S. 1983. Relationships between hydrophobicity and foaming characteristics of food proteins. J. Food Sci. 48: 588.

Voutsinas, L.P., Cheung, E., and Nakai, S. 1983a. Relationships of hydrophobicity to emulsifying properties of heat denatured proteins. J. Food Sci. 48: 26.

Voutsinas, L.P., Nakai, S., and Harwalker, V.R. 1983b. Relationships between protein hydrophobicity and thermal functional properties of food proteins. Can. Inst. Food Sci. Technol. J. 16: 185.

Voutsinas, L.P. and Nakai, S. 1983. A simple turbidimetric method for determining the fat binding capacity of proteins. J. Agric. Food Chem. 31: 58.

Weber, G. and Young, L.B. 1964. Fragmentation of bovine serum albumin by pepsin. I. The origin of the acid expansion of the albumin molecule. J. Biol. Chem. 239: 1415.

Wilson, K.J., Honeger, A., Stotzel, R.P., and Hughes, G.J. 1981. The behaviour of peptides on reverse-phase supports during high-pressure liquid chromatography. Biochem. J. 199: 31.

Wishnia, A. and Pinder, T.W. 1966. Hydrophobic interactions in proteins. The alkane binding site of β-lactoglobulins A and B. Biochemistry 5: 1534.

Wolf, W.J. 1970. Soybean proteins: Their functional, chemical, and physical properties. J. Agric. Food Chem. 18: 969.

Zittle, C.A. and Custer, J.H. 1963. Purification and some of the properties of α_s-casein and κ-casein. J. Diary Sci. 46: 1183.

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T.A. Roberts, G. Hobbs, J.H.B. Christian, and N. Skovgaard, Academic Press, London.

Enfors, S-O., Molin, G., and Ternstrom, A.M. 1979. Effect of packaging under carbon dioxide, nitrogen or air on the microbial flora of pork stored at 4°C. J. Appl. Bacteriol. 47: 197.

Erichsen, I. and Molin, G. 1981. Microbial flora of normal and high pH beef stored at 4°C in different gas environments. J. Food Prot. 44: 866.

Gardner, G.A. 1966. A selective medium for the enumeration of Microbacterium thermosphactum in meat and meat products. J. Appl. Bacteriol. 29: 455.

Grau, F.H. 1981. Role of pH, lactate, and anaerobiosis in controlling the growth of some fermentative gram-negative bacteria on beef. Appl. Environ. Microbiol. 42: 1043.

Grau, F.H. 1983. Microbial growth on fat and lean surfaces of vacuum-packaged chilled beef. J. Food Sci. 48: 326.

Hess, E., Ruosch, W., and Breer, C. 1980. Extending the shelf-life of prepacked fresh meat. Fleischwirtsch. 60: 1513.

Hugh, R. and Leifson, E. 1953. The taxonomic significance of fermentative vs oxidative metabolism of carbohydrates by various gram-negative bacteria. J. Bacteriol. 66: 24.

Mossel, D.A.A., Visser, M., and Mengerink, W.H.J. 1962. A comparison of media for the enumeration of moulds and yeasts in foods and beverages. Lab. Pract. 11: 109.

Patterson, J.T. and Gibbs, P.A. 1978. Some microbiological considerations applying to the conditioning, ageing and vacuum packaging of lamb. J. Food Technol. 13: 1.

Sander, E.H. and Soo, H-M. 1978. Increasing shelf-life by carbon dioxide treatment and low temperature storage of bulk pack fresh chickens packaged in nylon/surlyn film. J. Food Sci. 43: 1519.

Seideman, S.C., Smith, G.C., Carpenter, Z.L., Dutson, T.R., and Dill, C.W. 1979. Modified gas atmospheres and changes in beef during storage. J. Food Sci. 44: 1036.

Shaw, B.G., Harding, C.D., and Taylor, A.A. 1980. The microbiology and storage stability of vacuum packed lamb. J. Food Technol. 15: 397.

Thornley, M.J. 1960. The differentiation of Pseudomonas from other gram-negative bacteria on the basis of arginine metabolism. J. Appl. Bacteriol. 23: 37.

Ms received 6/6/84; revised 10/16/84; accepted 10/17/84.

Distribution and Rate of Migration of Curing Ingredients (Nitrite, Salt, Glucose) in Pork Tissue as Affected by Electrical Stimulation

H. W. OCKERMAN and K. KWIATEK

─────────── ABSTRACT ───────────

The effect of electrical stimulation of pork carcasses on distribution and rate of migration of curing ingredients (nitrite, salt, glucose) was investigated. Sixty cylindrical triceps brachii muscle samples were prepared at 24 hr post-stimulation from three pigs (left side stimulated; right side control, not stimulated) and determination for nitrite, salt and glucose were made at 24, 48 and 72 hr post-curing at each of four cylindrical segment depths of 1.25 cm each. The analysis of variance indicated that electrical stimulation caused significant ($P < 0.01$) improvement in absorption and migration of curing ingredients (nitrite, salt, glucose) into all depths of the pork cylinder.

INTRODUCTION

ELECTRICAL STIMULATION has been reported to improve tenderness in beef, lamb and goats (Chrystall and Hagyard, 1975, 1976; Grusby et al., 1976; Gilbert and Davey, 1976; Gilbert et al., 1976; Davey et al., 1976; Smith et al., 1977; Savell et al., 1977; Sorinmade et al., 1978) as well as influencing other meat quality (flavor, lean color, heat ring, marbling, retail case life) parameters (Savell et al., 1979).

Pork tissue has not been intensively investigated in terms of the influence of electrical stimulation on meat quality but in the pork research work of Smith et al. (1980) Crenwelge et al. (1980) and Johnson et al. (1982) it was reported that electrical stimulation does not appear to improve quality, quality indication or palatability traits of pork muscle.

The effects of electrical stimulation have not previously been evaluated to any major extent in terms of the migration rate and distribution of curing ingredients in either beef or pork tissue. Ockerman and Dowiercial, (1980) stated that neither tumbling nor electrical stimulation had a significant effect on the levels of sodium nitrite or sodium chloride under the conditions of their research. They did, however, report improved distribution of curing ingredients, especially nitrite, by electrical stimulation and that could be a very valuable factor in the current attempts to shorten the curing time or to reduce the nitrite level in curing solution and cured meat.

The objectives of this study were to determine whether electrical stimulation influences the distribution and rate of migration of curing ingredients in pork tissue during the curing process.

MATERIALS & METHODS

THREE PIGS were conventionally slaughtered and the left side of each carcass was electrically stimulated after splitting within 45 min post-slaughter. The carcasses were stimulated using a High Voltage JASEC Electrical Meat Stimulator (JASEC, Inc., Attica, IN). Each treated side received 15 electrical impulses of 500V alternating current of 2.5 sec each in duration followed by 2.5 sec of no current. The other side (right) was used as a control (nonstimulated). The triceps brachii muscles were removed 24 hr postmortem

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from both sides of the chilled carcasses. Cylindrical samples of 1.5 cm in diameter and 5 cm in length (Parallel to muscle fibers) were prepared from the muscles using an electrical cork borer on the firmly chilled tissue. These cylindrical samples were tightly placed into plastic tubes (1.5 cm internal diameter X 9 cm in length) in an effort to prevent migration of curing solution between the sample and sides of the tube. This technique has many similarities to the procedure used by Wistreich et al. (1959, 1960) to study chlorine accumulation. Curing solution (2.5 ml) composed of 20% NaCl, 6% glucose and 0.16% sodium nitrite was added to the tubes above the samples. Both stimulated and nonstimulated samples in the tubes were held at 3 – 5°C and sampled at 24, 48, and 72 hr. No visual PSE tissue was noted in either the fresh or cured tissue of either the control or stimulated samples; however, these small samples were not an ideal model for this evaluation and only a small number of hogs are represented in this sample. At each sampling time, the excess cure above the sample was discarded and the cylindrical sample was removed from the tube and divided into four cylindrical segments of 1.25 cm in depth from the top (adjacent to curing solution) to the bottom of the sample. These segmented samples were each individually analyzed for nitrite level using the procedure described by Ockerman (1981) with two necessary changes: (1) samples were blended in a Stomacher Lab-Blender with 80 mL hot 70°C distilled water for 2 min and, after that, were quantitatively transferred into a 500 mL volumetric flask, (2) no mercuric chloride was added to the flask after 2 hr extraction in a water bath to avoid the addition of any extra chloride ions. Ockerman and Dowiercial (1980) have reported that elimination of merucric chloride did not affect the analysis for nitrite in cured tissue. In this way it was possible to use the same extract to determine content of nitrite, sodium chloride and glucose. Salt was analyzed by the Dicromat (R) (Diamond Crystal Salt Co., St. Clair, MI) procedure (Anonymous, 1977) which involved using the salt analyzer containing an electronic probe. Glucose was determined by the procedure described by Koniecko (1979) for meat products. Ten triceps brachii cylindrical samples were prepared for each treatment (stimulated and nonstimulated) and each time period (24, 48, 72 hr) and each segment (0 – 1.25, 1.26 – 2.50, 2.51 – 3.75, 3.76 – 5.0 cm) was analyzed for NaCl, $NaNO_2$ and glucose. This resulted in a total of 40 determinations for nitrite, salt and glucose for each treatment (stimulated vs nonstimulated) and for each period of time (24, 48, and 72 post cure).

Analysis of variance (Harvey, 1968) was used to determine the significance of stimulation, depth of sample, time and their interactions. Means were separated by Duncan Multiple Range Test (Ockerman, 1983).

RESULTS & DISCUSSION

THE RESULTS of migration and distribution of curing ingredients (nitrite, salt, glucose) in stimulated and nonstimulated pork tissue are shown in Fig. 1 – 6).

All two-way interactions (stimulation X time, stimulation X depth, time X depth) were significant ($P < 0.01$) for nitrite concentrations and Fig. 1 illustrates the stimulation X depth relationships. This suggests that nitrite concentration was increased at each depth by stimulation and the analysis of variance indicated that stimulation had a significant ($P < 0.01$) influence on this nitrite level. Fig. 2 shows the three-way interaction (stimulation X time X depth) for nitrite concentration and again illustrates the effect of stimulation, depth and linear time on the penetration rate. The differences in the levels of nitrite in each cylindrical sample depth between stimulated and nonstimulated samples were approximately the same (20 – 30 ppm)

during the total time period of curing, except the segment of sample adjacent (0 – 1.25 cm in depth) to the curing solution after the 24 hrs curing period where the levels did not differ significantly between stimulated and non-stimulated samples.

As shown in Fig. 3 and 4 electrical stimulation caused a significant increase ($P < 0.01$) in the NaCl concentration at all sample depths. Figure 3 illustrates the significant

($P < 0.01$) stimulation × depth interaction and the general shape of the pattern is similar to Fig. 1 for nitrite. Figure 4 shows the three-way interaction (stimulation × time × depth) for salt and it is similar to Fig. 2 for nitrite except there is a slightly greater difference in the salt concentration for stimulated and nonstimulated tissue at the 0 – 1.25 cm depth of 24 hr and the salt differences are not as great and are not significant in the 3.75 – 5.0 cm depth at any time period. This decreased influence of electrical stimulation for salt at greater depths may be due to the larger influence of osmotic pressure of salt due to its higher concentration. The analysis of variance indicated that electrical stimulation as well as linear time (24, 48 and 72 hr post cure) and depth of tissue had a highly significant ($P < 0.01$) influence on the salt concentrations.

Electrical stimulation also caused an increase in glucose penetration and concentrations for all depths of cylindrical segmented samples in comparison to nonstimulated tissues (Fig. 5, 6), and again the concentration of glucose (Fig. 5) is similar to the relative concentration of nitrite in Fig. 1. Fig. 6 illustrates the concentration of glucose for the stimulation × time × depth interaction. The pattern
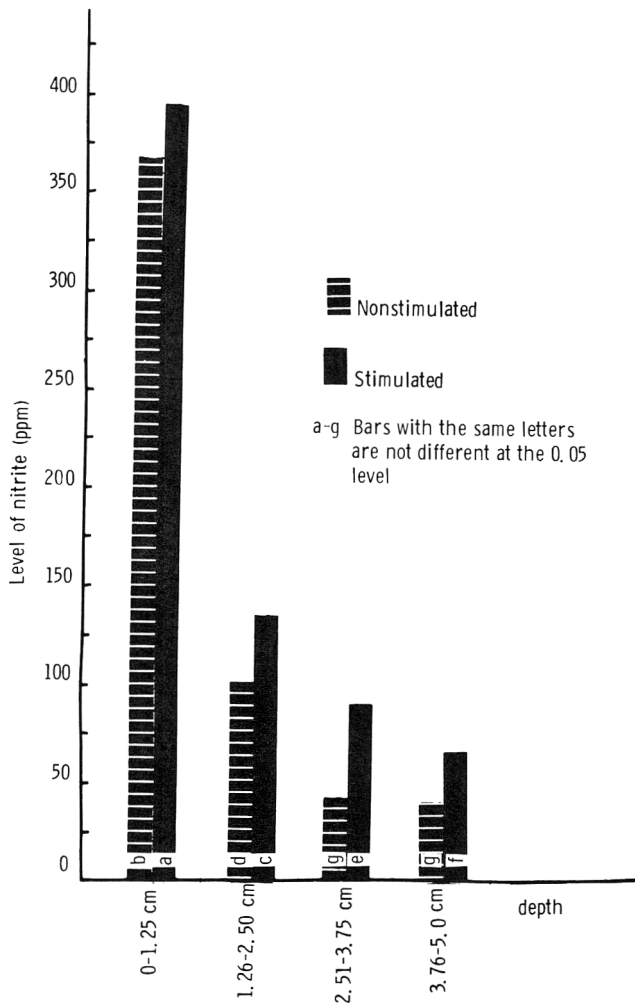

Fig. 1—Effect of electrical stimulation on the concentration of nitrite at several depths in pork tissue (n = 30 per bar).
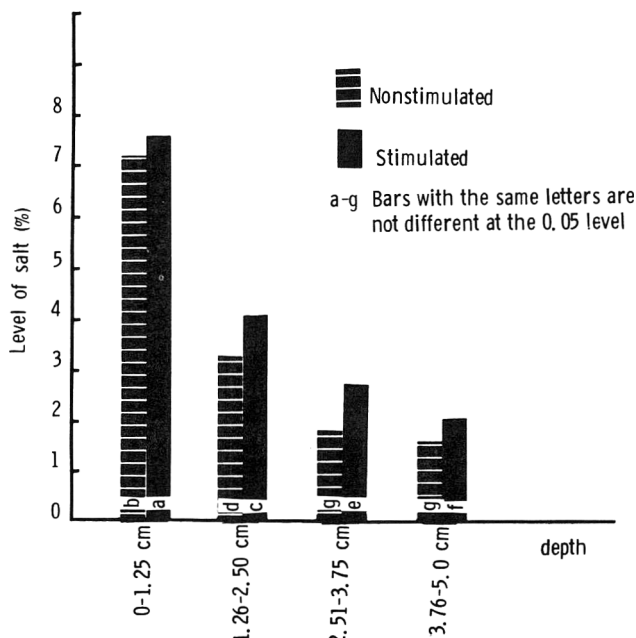

Fig. 3—Effect of electrical stimulation on the concentration of salt at several depths in pork tissue (n = 30 per bar).
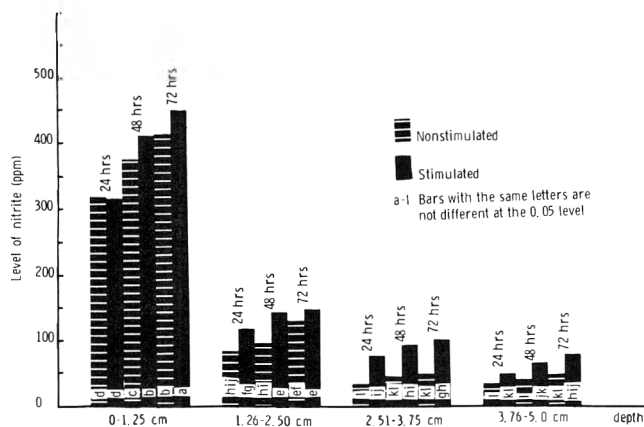

Fig. 2—Effect of of electrical stimulation, time and sample depth on the concentration of nitrite in pork tissue (n = 10 per bar).
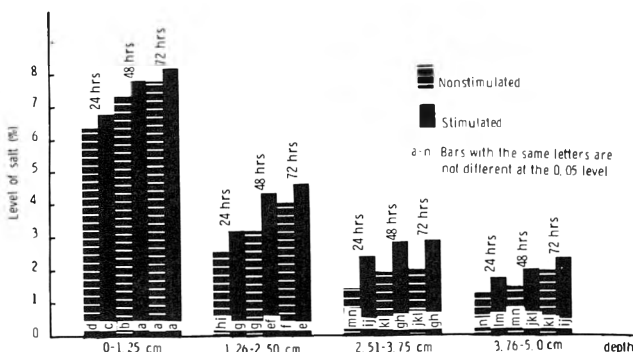

Fig. 4—Effect of electrical stimulation, time and sample depth on the concentration of salt in pork tissue (n = 10 per bar).

Fig. 5—Effect of electrical stimulation on the concentration of glucose at several depths in pork tissue (n = 30 per bar).

Fig. 6—Effect of electrical stimulation, time and sample depth on the concentration of glucose in pork tissue (n = 10 per bar).
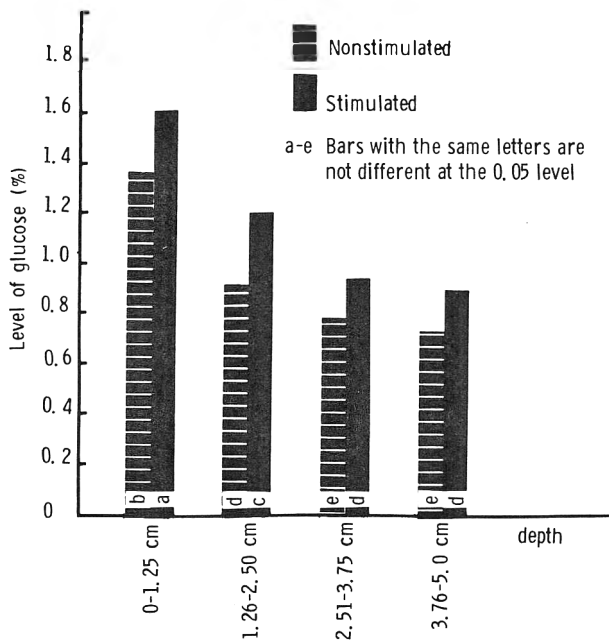
once again is similar to the nitrite (Fig. 2) and salt (Fig. 4) patterns previously observed.

This overall increase in migration and concentration of cure ingredients was probably influenced by the disruption of muscle tissue caused by electrical stimulation as would be expected and agrees in general with the report of Ockerman and Dowiercial (1980). In a normal curing operation the movement of curing pickle components would be caused by salt which diffuses inwards, forms a complex with the protein of meat which creates a higher osmotic pressure than the brine (Lawrie, 1979). When electrical stimulation is applied the distribution and migration of the cure ingredients may also be promoted by the disruption of the muscle sarcolemma, which may take place during stimulation (Savell et al., 1978; Sonaiya et al., 1982; Cross, 1978). This would promote the migration of curing ingredients both between muscle bundles and fibers and into those fibers with fragmented sarcolemma, resulting in a quicker and more uniform distribution of curing ingredients. It would be premature to recommend electrical stimulation of pork carcasses based on this research since it did not attempt to measure PSE conditions or loss of curing ingredients during heat processing.

REFERENCES

Anonymous, 1977. DICROMAT Salt Analyzer. Diamond Crystal Salt Co., St. Clair, MI.
Chrystall, B.B. and Hagyard, C.J. 1975. Accelerated conditioning of lamb. MIRINZ. Publication No. 470.
Chrystall, B.B. and Hagyard, C.J. 1976. Electrical stimulation and lamb tenderness. N.Z. J. Agri. Res. 19: 7.
Crenwelge, D.D., Terrell, R.N., Dutson, T.R., Smith, G.C., and Carpenter, Z.L. 1980. Electrical stimulation and postmortem chilling effects on pork quality. 26th European Meeting of Meat Research Workers, Colorado Springs, USA, 2: 108.
Cross, H.R. 1978. Effects of electrical stimulation on meat tissue and muscle properties—A review. J. Food Sci. 44: 509.
Davey, G.L., Gilbert, K.V., and Coarse, W.A. 1976. Carcass electrical stimulation to prevent cold-shortening toughness in beef. N.Z. J. Agr. Res. 19: 13.
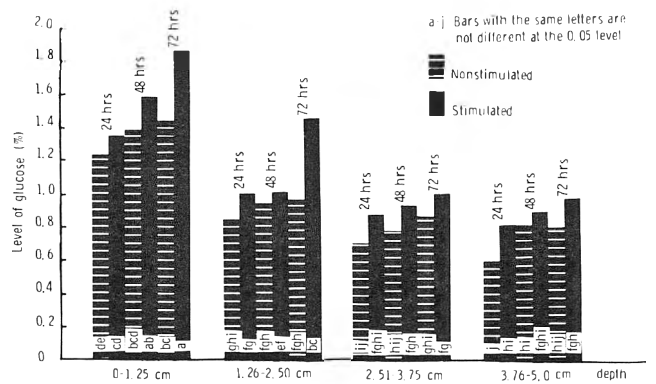
Gilbert K.V. and Davey, C.L. 1976. Carcass electrical stimulation and early boning of beef. N.Z. J. Agri. Res. 19: 249.
Gilbert, K.V., Davey, C.L., and Newton, K.G. 1976. Electrical stimulation and the hot-boning of beef. N.Z. J. Agri. Res. 20: 139.
Grusby, A.H., West, R.L., Carpenter, J.W., and Palmer, A.Z. 1976. Effect of electrical stimulation on tenderness. J. Anim. Sci. 42: 253.
Harvey, W.R. 1968. Least-squares analysis of data with unequal subclass numbers. Agri. Res. Serv. 20-8 USDA, Beltsville, MD.
Johnson, D.D., Savell, J.W., Weatherspoon, L., and Smith, G.C. 1982. Quality, palatability and weight loss of pork as affected by electrical stimulation. Meat Science 7: 43.
Koniecko, E.S. 1979. Handbook for meat chemists. Avery Publishing Group Inc., Wayne, NJ.
Lawrie, R.A. 1979. "Meat Science," Third ed. Pergamon Press. New York, NY.
Ockerman, H.W. 1981. "Quality Control of Postmortem Muscle Tissue." Animal Science Dept., The Ohio State Univ., Columbus, OH.
Ockerman, H.W. 1983. "Statistical Sampling Principles." Animal Science Dept., The Ohio State Univ., Columbus, OH.
Ockerman, H.W. and Dowiercial, R. 1980. Influence of tumbling and electrical stimulation on distribution and content of sodium nitrite and sodium chloride in bacon. J. Food Sci. 45: 1301.
Savell, J.W., Smith, G.C., Dutson, T.R., Carpenter, Z.L., and Suter, D.A. 1977. Effect of electrical stimulation on palatability of beef, lamb and goat meat. J. Food Sci. 42: 702.
Savell, J.W., Dutson, T.R., Smith, G.C., and Carpenter, Z.L. 1978. Structural changes in electrically stimulated beef muscle. J. Food Sci. 43: 1606.
Savell, J.W., Smith, G.C., Carpenter, Z.L., and Parrish, F.C. Jr. 1979. Influence of electrical stimulation on certain characteristics of heavyweight beef carcasses. J. Food Sci. 44: 911.
Smith, G.C., Dutson, T.R., Carpenter, Z.L., and Hosteller, R.L. 1977. Using electrical stimulation to tenderize meat. Proc. Meat Industry Research Conference 29: 147.
Smith, G.C., Savell, J.W., Dutson, T.R., Hosteller, R.L., Terrell, R.N., Murphey, C.E., and Carpenter, Z'L. 1980. Effects of electrical stimulation on beef, pork, lamb and goat meat. 26th European Meeting of Meat Research Workers. Colorado Springs, USA 2: 19.
Sonaiya, E.B., Stouffer, J.R., and Beerman, D.H. 1982. Electrical stimulation of mature cow carcasses and its effect on tenderness, myofibril protein degradation and fragmentation. J. Food Sci. 47: 1982.
Sorinmade, S.O., Cross, H.R., and Ono, K. 1978. The effect of electrical stimulation on lysosymal enzyme activity, pH decline and beef tenderness. 24th European Meat Conf. Kulmbach, Germany, 2:E 9: 1.
Wistreich, H.E., Morese, R.E., and Kenyon, L.J. 1959. Curing of ham: study of sodium chloride accumulation. 1. Food Technol. 13: 441.
Wistreich, H.E., Morse, R.E., and Kenyon, L.J. 1959. Curing of ham: A study of sodium chloride accumulation. 1. Food Technol. 14: 549.
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Descriptive Sensory Assessment of Beef Steaks by Category Scaling, Line Scaling and Magnitude Estimation

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ABSTRACT

Eighteen trained panelists evaluated the eating quality of a total of 54 cooked semimembranosus steaks from delay chilled and hot boned treatments using category scaling (CS), line scaling (LS) and magnitude estimation (ME) in different sequences. Treatment F-values showed that CS was most sensitive and LS was least sensitive in detecting differences in steak quality attributes. ME was as sensitive as CS to most treatment differences. For tenderness, correlations between each of the three evaluation techniques were high (r > 0.86). Significant correlations (r > 0.63) between panel tenderness assessments and shear data were also found. CS was most preferred and ME was least preferred by panelists. Panelists found ME the most difficult to use.

INTRODUCTION

MEAT SCIENTISTS routinely utilize trained descriptive panels to determine the effects of various production and processing treatments on the quality attributes of cooked meat. Category scales, generally employed for the descriptive sensory assessment of meat (AMSA, 1978), may have some shortcomings. Panelists may avoid using the ends of the scale and this "category end effect" may bias the ratings (O'Mahony, 1979). In addition, successive intervals along the scale may not be equal in subjective magnitude although data analyses are based on this assumption (Stevens and Galanter, 1957; Cloninger et al., 1976).

Semistructured line scales, which form the basis for "Quantitative Descriptive Analysis" (Stone et al., 1980), have been used by several meat researchers (Harries et al., 1972; Bouton et al., 1980) and have been reported to be more sensitive to product differences than structured scales (Baten, 1946). Reduced verbal anchoring may also minimize the problem of unequal intervals associated with the interpretation of descriptive terms (Jones et al., 1955).

Magnitude estimation, a ratio scaling technique, has become popular for food research (Moskowitz, 1983) but has not been widely utilized with meat (Segars et al., 1975; 1981). Use of magnitude estimation may eliminate potential biases of conventional (interval) scales because panelists are not limited to a fixed set of numbers. In addition, since instrumental measurements are calibrated in ratios, it may be advantageous to also have sensory measures on a ratio scale (Larmond, 1976).

Recommendations regarding the extent of panelist training for category scaling, line scaling and magnitude estimation differ. An 'expert' descriptive panel using category scales may receive three to four months of intensive training (AMSA, 1978), while panelists using line scales for quality evaluations usually receive about 10 hours of training (Zook and Wessman, 1977). With magnitude estimation,

it is felt that a conscientious person can make quantitative estimates of attributes after a few introductory sessions (Moskowitz, 1977). However, the use of magnitude estimation by trained rather than untrained panels may result in improved discrimination of textural attributes (Cardello et al., 1982a).

Comparative studies examining the use of category scaling (CS), line scaling (LS) and magnitude estimation (ME) in meat research are lacking. Therefore, this investigation was designed to compare the sensitivity and accuracy of these sensory evaluation techniques for the descriptive sensory assessment of beef, and to investigate statistical relationships between the three evaluation techniques and ease of scale usage.

MATERIALS & METHODS

Panelists

The panel consisted of 18 students and staff (17 females, 1 male; 18-35 years) in the Dept. of Foods & Nutrition at the Univ. of Alberta. Sixteen of the 18 were naive to sensory evaluation. The remaining panelists had some taste panel experience but had not served on a panel for several years.

Sixteen triangle tests were used to screen 28 potential panelists as described by Cross et al. (1978). Panelists were then intensively trained over a 12-wk period in the use of each evaluation technique, panel procedures and quality attribute definitions. The training time and experience provided for each evaluation technique was similar. A panel leader, equally experienced with each evaluation procedure, conducted all training sessions. The introduction and refinement of CS, LS and ME procedures followed a similar format. During training, panelists individually scored a wide variety of beef samples (different tender and less tender muscles from animals of various ages were cooked to varying degrees of doneness (61-75°C)) using each evaluation technique. For CS and LS, scores were recorded and then discussed in round table sessions. To avoid influencing panelists' choice of numbers when using ME, only relative differences between meat samples presented were discussed. In addition, panelists were oriented to ME using lines and shapes (Moskowitz, 1977) before evaluations of meat began.

Panel performance was evaluated twice during training following the procedures of Cross et al. (1978) and AMSA (1978). The panelists that participated were sensitive to sample differences, consistent over time and highly motivated.

Samples

A total of 18 semimembranosus (SM) roasts, nine from each of two postmortem processing treatments, were obtained from the Agriculture Canada Research Branch, Lacombe, Alberta. Details of breeding, management and postmortem handling of the animals were described by Jeremiah et al. (1984). Nine of the SM roasts were excised from delay chilled carcasses which had been held for the first 2 hr after slaughter at 10-15°C and then aged for 6 days at 2°C. The remaining nine roasts were hot boned 40 min postmortem and then aged for 6 days at 2°C. The roasts (distal end of the SM) were individually vacuum-packaged in barrier bags, stored (−30°C) for several months and then transported to the Univ. of Alberta.

Three adjacent 3.8 cm steaks were cut, perpendicular to the muscle fiber direction, from each frozen roast and then individually packaged and stored as described by Hawrysh and Wolfe (1983). The three steaks, from each of nine roasts per treatment, were assigned according to three 3 x 3 Latin square designs for preparation in each of three phases or time periods.

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Prior to use, each wrapped frozen steak was thawed for 3 hr at 22°C and then for 20 hr at 3°C. All steaks were individually roasted at 176°C to an internal temperature of 65°C. The internal temperature of each steak was monitored with two copper-constantan thermocouples connected to a Honeywell recording potentiometer. After cooling to 50°C, each cooked steak was weighed, wrapped in plastic and refrigerated for approximately 1 hr to facilitate sampling of the meat. Sampling procedures for sensory and objective measurements are described by Shand (1984). Total cooking time (min) was noted and the percentage cooking losses, based on the weight of each thawed steak, were calculated.

During sample preparation, the degree of doneness of each cooked steak was assessed by three experienced judges using a double-pointed category scale (1 = well-done, 5 = medium, 9 = rare).

Sensory evaluation

To minimize the effects of carryover, learning and motivational factors, panelists used each sensory evaluation technique sequentially, in different predetermined orders, in the three distinct phases of the study. In each phase, panelists evaluated one of three adjacent steaks from each of nine roasts per treatment using one of the three evaluation procedures. Prior to each phase, a short unidentified retraining period for each evaluation technique was held.

Panel sessions were held daily between 1030 hr and 1430 hr in an atmospherically controlled sensory panel room equipped with eight individual booths and white lights. Each panelist received two 1.3 cm cubes of beef from designated positions in each of four steaks (which represented two replications). The samples (in small covered glass jars) had been warmed to 50°C in double boiler systems on Salton Hotrays® assembled in each booth. Distilled water (21°C) was provided for rinsing between samples. Sample presentation was balanced for serving order, judge and replication.

Panelists assessed five descriptive attributes in each beef sample: initial tenderness (based on two chews with the molar teeth), overall tenderness, connective tissue amount, overall juiciness and flavor intensity. For CS, each panelist rated each attribute in the samples using an eight point descriptive category scale: 8 = extremely tender, juicy, meaty and no connective tissue; 1 = extremely tough, dry, weak and abundant connective tissue. Each panelist standardized his /her overall tenderness scores by the number of chews required to completely masticate a cube of beef. For LS, each panelist marked a vertical line across a 15 cm line at the point which best described his/her impression of each of the attributes in the beef cubes. The line scales were anchored 1.3 cm from each end with the appropriate descriptors (very tender-very tough; small amount connective tissue-large amount connective tissue; very juicy-very dry; very meaty-very weak). A value from 0.0-15.0 was assigned to each rating by converting the mark on the line to a numerical score. For the modulus free ME procedure, each panelist assigned any number (greater than 0) of his/her choice to describe each attribute in the first sample presented. In each successive sample, panelists assigned numbers in relation to those attributes in the previous sample. For ME, panelists were instructed to assign numbers to reflect the ratios of sensory intensity and that the ratio relationship of the numbers assigned was more important than the actual numbers assigned. The use of larger numbers indicated increased toughness, connective tissue amount, juiciness and meatiness.

Upon completion of the study, the panelists individually ranked the three evaluation techniques for ease of learning, effort required for sample evaluation and scale preference.

Objective measurements

Objective measurements of the cooked steaks were made on samples at 21°C taken from positions adjacent to those used for sensory evaluation. Eight to 10 cores (1.3 x 1.3 x 3.2 cm) per steak, cut parallel to the muscle fibers, were sheared once with a Warner Bratzler blade attached to the Ottawa Texture Measuring System (OTMS) using the procedure described by Hawrysh et al. (1979). The percentage press fluid in triplicate 0.5g samples from each steak was determined as described by Shand (1984). Hunter color measurements were made on samples from each cooked steak using procedures given by Hawrysh and Wolfe (1983).

Data analyses

Arithmetic means were calculated for CS and LS data; geometric means were calculated for ME. Least squares split-plot analyses of

variance were computed for the various factors under investigation. The magnitude estimates were transformed to logarithms for data analyses unless otherwise noted. However, it was not necessary to normalize ME data according to the procedures described by Powers et al. (1981) because panelist variation was removed in the analyses of variance procedures utilized.

Correlation and regression analyses were performed to assess linear and other mathematical functions for appropriate sensory data between each of the three evaluation techniques. In addition, for initial and overall tenderness, correlation and regression analyses were performed between each of the evaluation techniques and Warner Bratzler shear data. The functions evaluated included: linear (Y = a + bX), power (Y = aX^n), logarithmic (Y = a + blnX), hyperbolic (Y = a + b (1/X)) and parabolic (Y = a + bX + cX^2). For evaluation of the nonlinear functions, appropriate transformations of the data were made and linear regressions were computed. All correlations and regressions were computed across treatments rather than within treatments to allow an evaluation of relationships over the entire range of the data.

For the three evaluation techniques, the ranked data for selected criteria were converted to scores according to the method of Fisher and Yates (1942) as described by Larmond (1977) and then submitted to analyses of variance. Mean separation of ranks was done using Student Newman Keul's Multiple Range test (Steel and Torrie, 1980).

RESULTS & DISCUSSION

THE RAW WEIGHT, cooking time, final internal temperature and cooking losses for SM steaks (Table 1) show no differences attributable to postmortem treatment. In the present study, steaks took an average total cooking time of 56.8 min to reach 65°C and gave total, volatile and drip losses of 22.6%, 19.6% and 3.0%, respectively. Moore et al. (1980) roasted 3.8 cm SM steaks in a rotary gas oven at 177°C to 65°C in 55.2 min and obtained total, volatile and drip losses of 20.6%, 19.0% and 1.6%, respectively.

Data for the descriptive sensory assessments of delay chilled and hot boned steaks by CS, LS and ME are summarized in Table 2. When panelists employed CS, hot boned steaks were rated lower (P < 0.05) in initial tenderness and overall tenderness and higher (P < 0.01) in overall juiciness than comparable delay chilled steaks. With the LS technique, there were no significant differences in the quality attributes due to postmortem treatment. Using ME, panelists scored the hot boned steaks lower (P < 0.05) in overall tenderness and higher (P < 0.01) in juiciness than comparable delay chilled steaks.

Although there were differences in the levels of statistical significance, the direction and extent of the difference between mean scores assigned to each of the quality attributes in the delay chilled and hot boned steaks were generally similar for CS, LS and ME (Table 2). For each evaluation technique, differences between steak treatment means tended to be large for initial tenderness and overall tenderness, intermediate for amount of connective tissue and juiciness, and slight for flavor intensity.

Table 1—Means and standard errors for raw weight, cooking time, final internal temperature and cooking losses for semimembranosus steaks from delay chilled and hot boned treatments

Measurement	Postmortem treatment		SEM[a]
	Delay chilled	Hot boned	
Raw weight, g	457.7	449.6	52.31
Cooking time, min	55.3	58.4	2.75
Final internal temperature, °C	65.1	65.1	0.16
Cooking losses, %			
Total	23.1	22.1	1.29
Volatile	20.1	19.2	1.01
Drip	3.0	2.9	0.72

[a] Standard error of the means.

Table 2—Means and standard errors for category scaling, line scaling and magnitude estimation of semimembranosus steaks from delay chilled and hot boned treatments

Attribute	Postmortem treatment		
	Delay chilled	Hot boned	SEM[a]
Category scale[b]			
Initial tenderness	5.4	4.5	0.26*
Overall tenderness	5.1	4.2	0.23*
Connective tissue amount	4.9	4.6	0.12
Overall juiciness	4.7	5.0	0.05**
Flavor intensity	5.0	5.0	0.10
Line scale[c]			
Initial tenderness	9.8	8.2	0.50
Overall tenderness	8.9	7.6	0.42
Connective tissue amount	9.7	9.2	0.36
Overall juiciness	7.7	8.3	0.25
Flavor intensity	7.9	8.0	0.23
Magnitude estimation[d]			
Initial tenderness	13.7	15.5	(0.034)[e]
Overall tenderness	16.3	20.0	(0.023)*
Connective tissue amount	10.0	11.5	(0.023)
Overall juiciness	12.3	14.4	(0.014)**
Flavor intensity	13.9	14.4	(0.012)

[a] Standard error of the means.
[b] Maximum score = 8. Higher values indicate increased tenderness, juiciness, flavor intensity and decreased connective tissue amount.
[c] Maximum score = 15. Higher values indicate increased tenderness, juiciness, flavor intensity and decreased connective tissue amount.
[d] Modulus free scoring. Higher values indicate increased toughness, connective tissue amount, juiciness and flavor intensity.
[e] Standard error expressed as log 10 value.
*,** Significant at P < 0.05 and P < 0.01, respectively.

Table 3—Means and standard errors for objective measurements and degree of doneness for semimembranosus steaks from delay chilled and hot boned treatments

Measurement	Postmortem treatment		
	Delay chilled	Hot boned	SEM[a]
OTMS-Warner Bratzler blade, kg	7.7	8.7	0.66
Degree of doneness[b]	4.9	5.6	0.14 **
Hunter L	44.9	42.6	1.93
a	6.8	8.4	0.09 ***
b	10.4	10.5	0.13
Press fluid, %	36.5	40.1	0.61 **

[a] Standard error of the means.
[b] Doneness scale: 1 = well-done, 5 = medium, and 9 = rare.
,* Significant at P < 0.01 and P < 0.001, respectively.

Table 4—Treatment F-values from analyses of variance of quality attributes in semimembranosus steaks from category scaling, line scaling, and magnitude estimation

Attribute	Treatment F-values		
	Category scaling	Line scaling	Magnitude estimation
Initial tenderness	6.63*	4.97	1.22
Overall tenderness	7.33*	5.11	7.30*
Connective tissue amount	4.33	1.21	3.92
Overall juiciness	13.55**	3.03	12.26**
Flavor intensity	0.00	0.10	0.82

*,** Significant at P < 0.05 and P < 0.01, respectively (df = 1, 8)

Table 5—Pearson correlation coefficients (r) between panel assessments from each evaluation technique[a]

Attribute	r
Initial tenderness	
CS vs LS	0.93***
CS vs ME	−0.86***
LS vs ME	−0.91***
Overall tenderness	
CS vs LS	0.93***
CS vs ME	−0.92***
LS vs ME	−0.92***
Connective tissue amount	
CS vs LS	0.54*
CS vs ME	−0.34
LS vs ME	−0.53*
Overall juiciness	
CS vs LS	0.44*
CS vs ME	0.74***
LS vs ME	0.65**
Flavor intensity	
CS vs LS	0.37
CS vs ME	0.59**
LS vs ME	0.36

[a] Category scaling (CS), line scaling (LS) and magnitude estimation (ME).
*,**,*** Significant at P < 0.05, P < 0.01 and P < 0.001, respectively.

Sensory assessments of tenderness tend to be supported by Warner Bratzler shear data (Table 3). Although the difference was not statistically significant, the average shear force value for hot boned steaks was higher than that of comparable delay chilled steaks. Since steaks from the delay chilled and hot boned treatments attained the same average internal temperature of 65.1°C (range, 64.8-65.5°), juiciness was not expected to differ between the treatments. However, the significant differences for juiciness observed in Table 2 are supported by objective measurements (Table 3). Visual evaluation of steak doneness indicated that the hot boned steaks were more rare (P < 0.01) than comparable delay chilled steaks. Although Hunter L (lightness) and b (yellowness) values for both treatments were similar, the Hunter a (redness) value for hot boned steaks was higher (P < 0.001) than that of comparable delay chilled steaks. In addition, a greater (P < 0.01) percentage of press fluid was released from hot boned steaks than from comparable delay chilled steaks.

Jeremiah et al. (1984), using similar SM steaks but a greater number of replications than in the present study, reported that hot boned steaks were lower (P < 0.05) in initial tenderness and overall tenderness and higher (P < 0.05) in connective tissue than their delay chilled counterparts. Flavor scores between postmortem treatments did not differ significantly. In contrast to results of the present study, Jeremiah et al. (1984) did not find significant differences in juiciness due to postmortem treatment.

Treatment F-values were used as a measure of scale sensitivity (Moskowitz, 1982). CS was most sensitive to treatment differences (Table 4) as indicated by the three significant F-values obtained for initial tenderness, overall tenderness and juiciness. For the LS technique, no significant treatment F-values were determined. For ME, treatment F-values for overall tenderness and juiciness were significant and similar to those obtained by CS; however, the F-value for initial tenderness was not statistically significant. Difficulties with the ME procedure may have con-

tributed to panelists' inability to discriminate differences in initial tenderness between the two postmortem treatments. Because of the time and effort required for sample mastication, panelists noted difficulty in remembering their im-

Table 6—Coefficients of determination[a] (R^2) for initial and overall tenderness between each of the three evaluation techniques[b] fitted to five mathematical functions

Comparison Y vs X	Mathematical function				
	Linear (Y = a + bX)	Power (Y = aX[n])	Logarithmic (Y = a + blnX)	Hyperbolic (Y = a + b (1/X))	Parabolic (Y = a + bX + cX^2)
Initial tenderness					
CS vs LS	0.86	0.89	0.85	0.81	0.87
LS vs CS	0.86	0.89	0.89	0.90	0.91
CS vs ME	0.74	0.76	0.77	0.78	0.78
ME vs CS	0.74	0.76	0.74	0.73	0.74
LS vs ME	0.82	0.79	0.82	0.79	0.83
ME vs LS	0.82	0.79	0.81	0.79	0.83
Overall tenderness					
CS vs LS	0.86	0.86	0.83	0.78	0.90
LS vs CS	0.86	0.86	0.89	0.90	0.90
CS vs ME	0.84	0.85	0.85	0.86	0.86
ME vs CS	0.84	0.85	0.84	0.82	0.84
LS vs ME	0.84	0.79	0.84	0.83	0.84
ME vs LS	0.84	0.79	0.80	0.75	0.89

[a] All coefficients of determination were significant at $P < 0.001$.
[b] Category scaling (CS), line scaling (LS) and magnitude estimation (ME).

Table 7—Pearson correlation coefficients (r) between panel tenderness assessments from each evaluation technique[a] and Warner Bratzler shear data

Attribute	r
Initial tenderness	
CS	−0.71***
LS	−0.68***
ME	0.63**
Overall tenderness	
CS	−0.68***
LS	−0.69***
ME	0.70***

[a] Category scaling (CS), line scaling (LS) and magnitude estimation (ME).
,* Significant at $P < 0.01$ and $P < 0.001$, respectively.

pressions of one sample relative to another. However, Moskowitz (1983) observed that panelists develop their own internal frame of reference for assigning scores to each sample and thus may not need to remember attributes in successive samples.

The panelists' inability to distinguish treatment differences by the LS technique was unexpected. Stone et al. (1980) have reported success with line scales for "Quantitative Descriptive Analysis;" however, few studies have compared the sensitivity of the LS technique to other evaluation procedures. Although graphic (line) scaling allows a panelist to discriminate as finely as desired, this scaling technique does not force such a judgement (Symonds, 1924). Raffensperger et al. (1956) scored the tenderness of beef cuts from different grades using a nine point category scale and a modified line scale (nine divisions were marked) and obtained similar results using each method. However, for assessments of apple quality, Baten (1946) reported that the LS technique resulted in larger t-values than the CS technique. In addition, Giovanni and Pangborn (1983) obtained larger treatment F-values for panelists' evaluations of intensities of fat in milk and sucrose in lemonade by the LS technique than by ME.

Comparisons of CS and ME procedures have frequently been made by untrained panels for hedonic evaluations of food products (Moskowitz and Sidel, 1971; Moskowitz, 1982). However, few studies using trained panelists have compared CS and ME for the descriptive assessment of food

attributes. McDaniel and Sawyer (1981) used nine point CS and modulus free ME to score the intensity of 19 descriptive profile terms of whiskey sour formulations. ME yielded a similar number of significant differences to CS (McDaniel and Sawyer, 1981).

Correlation coefficients between each of the evaluation techniques (Table 5) for initial and overall tenderness were highly significant and indicated strong linear relationships between scores assigned by judges using CS, LS and ME. The assignment of higher numbers indicated greater tenderness for both CS and LS. High numbers signified toughness with ME. Thus the positive and negative relationships are as expected. For the remaining attributes, the correlations between each of the three techniques, while sometimes significant, were moderate to low. Sample heterogeneity, especially for connective tissue amount, and a narrow stimulus range, particularly for juiciness and flavor intensity, may have contributed to the low correlations obtained (Szczesniak, 1968; Cross et al., 1978).

Coefficients of determination (Table 6) obtained for tenderness assessments from CS, LS and ME fitted to each of five mathematical functions were similar. None of the mathematical functions provided a consistently superior fit of the data. For the parabolic functions between CS and ME tenderness data, the quadratic (X^2) term did not make a significant contribution to the function. Thus, for initial and overall tenderness, the relationships between CS and ME data are not curvilinear. In contrast, Cardello et al. (1982b) rescaled six standard texture scales using ME and found that the CS data was concave downward relative to the ME data. This indicated that at higher intensities of each attribute evaluated, CS was less sensitive than ME to differences in these attributes. In the present study, however, data for the evaluation of beef samples using CS did not cover the entire stimulus range of the scale and thus may have influenced the shape of the resulting functions.

Regression analyses of overall tenderness data between each of the three evaluation techniques yielded the following linear regression equations: for CS and LS, $Y = 0.16 + 0.55X$ ($r^2 = 0.86$); for CS and ME, $Y = 8.45 − 0.20X$ ($r^2 = 0.84$); and for LS and ME, $Y = 14.65 − 0.35X$ ($r^2 = 0.84$), respectively.

Correlations of sensory scores and mechanical measurements have been used as an indicator of panel accuracy (Hovenden et al., 1979). Correlations of tenderness data with Warner Bratzler shear values (Table 7) yielded similar significant r-values. However, these correlations only

Table 8—Coefficients of determination[a] (R^2) between panel tenderness assessments from each evaluation technique[b] and Warner Bratzler shear data fitted to five mathematical functions

	Mathematical function				
Attribute	Linear $(Y = a + bX)$	Power $(Y = aX^n)$	Logarithmic $(Y = a + b \ln X)$	Hyperbolic $(Y = a + b (1/X))$	Parabolic $(Y = a + bX + cX^2)$
Initial tenderness					
CS	0.51	0.53	0.51	0.50	0.51
LS	0.47	0.47	0.46	0.44	0.47
ME	0.40	0.37	0.39	0.37	0.40
Overall tenderness					
CS	0.46	0.47	0.46	0.45	0.46
LS	0.48	0.48	0.47	0.44	0.49
ME	0.49	0.47	0.48	0.47	0.49

[a] Coefficients of determination $\geq$ 0.46 were significant at $P < 0.001$; coefficients of determination $\geq$ 0.33 were significant at $P < 0.01$.
[b] Category scaling (CS), line scaling (LS) and magnitude estimation (ME).

accounted for 40-51% of the variation in the data. The high unexplained variation in the data may be a reflection of the lack of sensitivity of the Warner Bratzler shear to structural components that influence taste panel assessments. Gullett et al. (1983) reported that shear values may not be related to the panelists' perceptions of meat tenderness.

Similar coefficients of determination (Table 8) between sensory assessments of tenderness and shear data were determined for each of the five mathematical functions. Thus, the CS, LS and ME data can be explained equally well by each of these functions. Furthermore, linear, logarithmic, hyperbolic and parabolic functions described ME data as well as the more traditionally used power function. This finding agrees with a recent report by Giovanni and Pangborn (1983). For initial tenderness and overall tenderness, the power functions relating ME and shear data yielded exponents of 1.13 and 1.05, respectively. Because of data variability, the standard errors for initial tenderness (0.37) and overall tenderness (0.28) are large. Thus these exponents may not be reliable indicators of sensory response. Segars et al. (1975) reported that exponents relating textural properties of meat to an instrumental measure (punch and die shear device) ranged from 1.8 to 2.6. However, comparison of exponents between studies is difficult because exponents may be dependent on stimulus range and response range (Birnbaum, 1982).

Panelists ranked the evaluation techniques for selected criteria (Table 9). CS and LS were easier to learn and required less effort for sample evalution than ME. In addition, panelists preferred CS over both LS and ME. LS was ranked intermediate in preference and ME was least preferred. The effect of scale preference on motivation is unknown. If a panelist is not comfortable with the task, sensory performance may be affected. However, Baten (1946) reported that even though panelists preferred the CS technique to the LS technique, their discrimination of differences in apple varieties was greater with LS than with CS.

Panelists stated that the principle of ME was easy to understand but that its application to meat was difficult. Generally, the descriptive sensory assessment of beef involves the simultaneous evaluation of several characteristics. This type of assessment may be more difficult with ME than with either CS or LS, and may result in decreased sensitivity to treatment differences. Segars et al. (1981) modified their ME procedure for irradiated beef, ham and poultry rolls in order to reduce memory interference and to make ME evaluations easier for the panelists (Cardello, personal communication). Originally, panelists evaluated four attributes in each sample in the series presented; in a later phase of the investigation, panelists evaluated a

Table 9—Mean values for the ranking of selected criteria for category scaling (CS), line scaling (LS) and magnitude estimation (ME) by the 18 panelists

	Evaluation technique		
Criteria[d]	Category scaling	Line scaling	Magnitude estimation
Ease of learning	1.4[b]	1.6[b]	3.0[a] ***
Effort for sample evaluation	1.6[b]	1.6[b]	2.8[a] ***
Scale preference	1.4[c]	2.1[b]	2.6[a] ***

[a,b,c] Means within the same row sharing a common superscript are not significantly different at $P < 0.05$.
[d] Ranking based on: 1 = easiest, least effort and most preferred; 3 = most difficult, most effort and least preferred.
*** Significant at $P < 0.001$.

separate series of samples for each of three attributes (Segars et al., 1981).

CONCLUSIONS

RESULTS of this work indicate that for the descriptive sensory assessment of SM steaks, CS was most sensitive to treatment differences in steak attributes and was preferred by the trained panelists. Panelist evaluations by the LS technique did not result in treatment differences for any of the attributes scored. However, the LS technique was considered easy to learn and required little effort for sample evaluation. Although panelists found that the ME technique demanded the most effort for sample evaluation and was least preferred, ME was as sensitive as the CS technique to most treatment differences. To optimize the potential of the ME procedure for the descriptive assessments of cooked beef, some procedural modifications may be needed.

REFERENCES

AMSA. 1978. "Guidelines for Cookery and Sensory Evaluation of Meat." Am. Meat Sci. Assn. and Natl. Live Stock and Meat Board, Chicago, IL.
Baten, W.D. 1946. Organoleptic tests pertaining to apples and pears. Food Res. 11: 84.
Birnbaum, M.H. 1982. Problems with so-called "direct" scaling. In "Selected Sensory Methods: Problems and Approaches to Hedonics," (Ed.) J.T. Kuznicki, R.A. Johnson and A.F. Rutkiewic, p. 34. ASTM STP 773, Philadelphia, PA.
Bouton, P.E. Ford, A.L., Harris, P.V., and Shaw, F.D. 1980. Electrical stimulation of beef sides. Meat Sci. 4: 145.
Cardello, A.V. 1981. personal communication.
Cardello, A.V., Maller, O., Kapsalis, J.G., Segars, R.A., Sawyer, F.M., Murphy, C., and Moskowitz, H.R. 1982a. Perception of texture by trained and consumer panelists. J. Food Sci. 47: 1186.
Cardello, A.V., Matas, A., and Sweeney, J. 1982b. A research note. The standard scales of texture: Rescaling by magnitude estaimation. J. Food Sci. 47: 1738.

Cloninger, M.R., Baldwin, R.E., and Krause, G.F. 1976. Analysis of sensory rating scales. J. Food Sci. 41: 1225.

Cross, H.R., Moen, R., and Stanfield, M.S. 1978. Training and testing of judges for sensory analysis of meat quality. Food Technol. 32(7): 48.

Fisher, R.A. and Yates, F. 1949. "Statistical Tables for Biological, Agricultural and Medical Research," 3rd ed., Oliver and Boyd Ltd., London, England.

Giovanni, M.E. and Pangborn, R.M. 1983. Measurement of taste intensity and degree of liking of beverages by graphic scales and magnitude estimation. J. Food Sci. 48: 1175.

Gullett, E.A., Rowe, D.L., and Hines, R.J. 1983. Tenderness parameters in sensory evaluation of meat. Can. Inst. Food Sci. Technol. J. 16(3): xii (abstract).

Harries, J.M., Rhodes, D.N., and Chrystall, B.B. 1972. Meat texture. 1. Subjective assessment of the texture of cooked beef. J. Texture Studies 3: 101.

Hawrysh, Z.J., Price, M.A., and Berg, R.T. 1979. The effect of conventional and microwave cooking on the eating quality of beef from bulls of three differing breed-types. Can. Inst. Food Sci. Technol. J. 12(2): 78.

Hawrysh, Z.J. and Wolfe, F.H. 1983. Effects of low voltage electrical stimulation on mature cow carcasses: Part 1—selected quality attributes of beef. Meat Sci. 8: 119.

Hovenden, J.E., Dutson, T.R., Hostetler, R.L., and Carpenter, Z.L. 1979. Variation and repeatability of an untrained beef sensory panel. J. Food Sci. 44: 1598.

Jeremiah, L.E., Martin, A.H., and Murray, A.C. 1984. The effects of various postmortem treatments on certain physical and sensory properties of three different bovine muscles. Meat Sci. In press.

Jones, L.V., Peryam, D.R., and Thurstone, L.L. 1955. Development of a scale for measuring soldiers' food preferences. Food Res. 20: 512.

Larmond, E. 1976. Textural measurement in meat by sensory evaluation. J. Texture Studies 7: 87.

Larmond, E. 1977. "Laboratory Methods for Sensory Evaluation of Food." Research Branch, Canada Department of Agriculture, Publication 1637.

McDaniel, M.R. and Sawyer, F.M. 1981. Descriptive analysis of whiskey sour formulations: Magnitude estimation versus a 9-point category scale. J. Food Sci. 46: 178.

Moore, L.J., Harrison, D.L., and Dayton, A.D. 1980. Differences among top round steaks cooked by dry or moist heat in a conventional or a microwave oven. J. Food Sci. 45. 777.

Moskowitz H.R. 1977. Magnitude estimation: Notes on what, how, when, and why to use it. J. Food Qual. 1: 195.

Moskowitz, H.R. 1982. Utilitarian benefits of magnitude estimation scaling for testing product acceptability. In "Selected Sensory Methods: Problems and Approaches to Hedonics," (Ed.) J.T. Kuznicki, R.A. Johnson and A.F. Rutkiewic, p. 11. ASTM STP 773, Philadelphia, PA.

Moskowitz, H.R. 1983. "Product Testing and Sensory Evaluation of Foods. Marketing and R & D Approaches." Food & Nutrition Press Inc., Westport, CT.

Moskowitz, H.R. and Sidel, J.L. 1971 Magnitude and hedonic scales of food acceptability. J. Food Sci. 36: 677.

O'Mahony, M. 1979. Psychophysical aspects of sensory analysis of dairy products: A critique. J. Dairy Sci. 62: 1954.

Powers, J.J., Warren, C.B., and Masurat, T. 1981. Collaborative trials involving three methods of normalizing magnitude estimations. Lebensm.-Wiss. u. -Technol. 14: 86.

Raffensperger, E.L., Peryam, D.R., and Wood K.R. 1956. Development of a scale for grading toughness-tenderness in beef. Food Technol. 10: 627.

Segars, R.A., Hamel, R.G., Kapsalis, J.G., and Kluter, R.A. 1975. A punch and die test cell for determining the textural qualities of meat. J. Texture Studies 6: 211.

Segars, R.A., Cardello, A.V., and Cohen, J.S. 1981. Objective and subjective texture evaluation of irradiation sterilized meat products. J. Food Sci. 46: 999.

Shand, P.J. 1984. Descriptive sensory assessment of beef steaks by category scaling, line scaling and magnitude estimation. M.Sc. thesis, Univ. of Alberta.

Steel, R.G.D. and Torrie, J.H. 1980. "Principles and Procedures of Statistics. A Biometrical Approach." 2nd ed. McGraw-Hill Book Co., Toronto.

Stevens, S.S. and Galanter, E.H. 1957. Ratio scales and category scales for a dozen perceptual continua. J. Exp. Psychol. 54: 377.

Stone, H., Sidel, J.L., and Bloomquist, J. 1980. Quantitative descriptive analysis. Cereal Foods World 25: 642.

Symonds, P.M. 1924. On the loss of reliability in rating due to coarseness of the scale. J. Exp. Psychol. 7: 456.

Szczesniak, A.S. 1968. Correlations between objective and sensory texture measurements. Food Technol. 22(8): 49.

Zook, K. and Wessman, C. 1977. The selection and use of judges for descriptive panels. Food Technol. 31(11): 56.

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Effect of Maillard Reaction Products on the Stability of Minced Herring in Frozen Storage

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————————ABSTRACT————————

Crude and partially purified Maillard reaction products (MRP) from arginine and xylose, and crude MRP from histidine and glucose, were tested as antioxidants in a mince of fresh herring (*Clupea harengus*). Mince portions containing various concentrations of MRP were stored at −20°C. Samples were withdrawn at 0, 2, 5, 8, 12, 16, 10, 24, and 28 wk of storage and subjected to sensory evaluation, GC and GC/MS analysis of headspace volatiles, and peroxide value determination. Substantial oxidation was shown with all measuring methods, and no significant inhibitory effect was found for any Maillard additive throughout the 28-wk period.

INTRODUCTION

FATTY FISH, such as herring and mackerel, are extremely susceptible to lipid oxidation because of their high content of polyunsaturated fatty acids. The highly unsaturated acids C20:5 and C22:6 constitute more than 10% of the total fatty acids in herring (Ackman, 1980). Lipid oxidation, thus becomes the spoiling mechanism that limits the storage time for such fatty fish. Mincing of the fish enhances susceptibility to oxidation, since protective membrane systems then are destroyed and contact is facilitated among lipids, oxygen, and lipid oxidation catalysts in the fish tissue. There are three methods for retarding oxidation in fish products: lowering the storage temperature (Hardy and Smith, 1976), excluding oxygen (Hardy and Smith, 1976; Bilinski et al., 1979; Smith et al., 1980), and adding antioxidants. A wide range of antioxidative compounds, including sodium erythorbate, ascorbic acid, glutamates, EDTA, and several phenolic compounds, have been tried in fish and fish products (Hiremath, 1973; Deng et al., 1977; Moledina et al., 1977; Bilinski et al., 1979). The effectiveness of additives depends on the type of fish product and on the likelihood of obtaining an even distribution in the product. In general, water-soluble antioxidants have been more useful than lipid-soluble ones, probably owing to better distribution.

Maillard reaction products (MRP) from amino acids and sugars have not been tested in fish products, but they have been shown to possess antioxidative properties in model systems (Lingnert and Eriksson, 1981) and have also been successfully used in foods, such as frozen sausage (Lingnert and Lundgren, 1980), cookies (Lingnert, 1980), and milk powder (Hall and Lingnert, 1984). In all these food experiments, unpurified MRP from histidine and glucose were used. The objective of this study was to determine the effectiveness of water-soluble MRP at inhibiting lipid oxidation in minced herring during frozen storage.

MATERIALS & METHODS

Synthesis of MRP

MRP were prepared from the combinations arginine-xylose

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and histidine-glucose. The arginine-xylose product was prepared by refluxing a solution of 4.0 mol L-arginine monohydrochloride (Ajinimoto Co. Inc., Japan) and 2.0 mol D-xylose (Merck, GFR) in 2.0L distilled water for 20 hr. The crude reaction mixture obtained is designated at AXC. One liter AXC was partially purified (Lingnert et al., 1983) by dialyzing three times (24 hr each) against 17.5L degassed, N2-saturated, distilled water with Spectrapor 6 dialysis tubing (Spectrum Medical Industries Inc., Los Angeles, CA) with a molecular weight cut-off of 1000 daltons. The retentate obtained is designated as AX.

The histidine-glucose product was similarly prepared from 1.0 mol L-histidine monohydrochloride monohydrate (Ajinimoto) and 0.5 mol D-glucose (Fluka, Switzerland) in 1.0L distilled water. Before heating the mixture, it was adjusted to pH 7.0 with KOH. The crude reaction mixture is designated as HGC.

AX, AXC, and HGC were saturated with nitrogen and stored at +4°C until use.

Preparation of mince

Sixty kg freshly caught (less than 24 hr, kept in ice), deboned and skinned herring was minced and divided into six 9-kg portions to which was added 450 mL distilled water or 450 mL of one of the following solutions: AX (0.2 mg/mL), AX (2.0 mg/mL), AX (20 mg/mL), AXC (20 mg/mL), and HGC (20 mg/mL). The final experimental samples, therefore, were a control (I) and mince containing 0.001% AX (II), 0.01% AX (III), 0.1% AX (IV), 0.1% AXC (V), and 0.1% HGC (VI).

One hundred round patties (65g each) were formed from each portion, immediately frozen in a blast freezer at −30°C, and individually packed in laminated aluminum pouches. The pouches were sealed and stored at −20°C. Approximately 36 hr elapsed from the time the fish was caught until the final portion was frozen.

Seven patties of each experimental sample were withdrawn at 0, 2, 5, 8, 12, 16, 20, 24, and 28 wk of storage and subjected to sensory evaluation, GC and GC/MS analysis of volatile compounds, and peroxide value determination. Three of the samples from each storage time were transferred to storage at −80°C to be used for sensory evaluation at the end of the storage experiment.

Sensory evaluation

The patties were thawed by placing the sealed pouches in a 20°C water bath for approximately 1 hr. Each patty was then divided into ten identical portions (6.5g each) which were placed in covered cups (250 mL), equilibrated to room temperature for approximately 1 hr, and presented to the judges for evaluation.

Total odor intensity and intensity of individual odor characteristics (fresh herring, salted herring, rancid herring, sourness, and sweetness) were evaluated by a panel of nine judges who had previously participated in sensory evaluation of rancid foods, but were not trained judges of herring. The intensity scale consisted of a 10-cm-long straight line with the anchor points "little" and "much" 1 cm from the left and right endpoints, respectively. The judges were allowed to indicate intensity anywhere on the line. The evaluations were transformed into scores from 0.0 – 10.0 with a digitizer (Hewlett Packard model No. 9111A), and these scores were subjected to statistical analyses described below.

During introductory sessions, the panelists were presented with the experimental samples as well as samples of frozen herring (without MRP) of various storage times. They were also given very fresh herring (within 24 hr after catch, and kept near 0°C) to become acquainted with the odor of such herring. The panel chose the odor terms mentioned above as adequate to describe the experimental samples. Total odor intensity was defined as odor intensity without regard for its character. Fresh herring was defined as already mentioned, and rancid herring by the odor of herring stored in air ("old fish liver oil").

Samples perceived as different from fresh herring, but not yet rancid, were described as having the odor of "salted herring," a common food in Sweden. Finally, the panelists found the odor of the samples somewhat "sweet" and "sour".

Experimental designs. Differences among the six samples were studied at each withdrawal time using experimental design A described below. Combining results into one statistical analysis permitted indirect study of the effect of storage time on a particular sample. However, this indirect procedure was considered insensitive to small changes among the samples. Therefore, Design B was used shortly after the termination of the storage experiment to confirm or invalidate the conclusions drawn from the series of experiments of Design A. Design B involved a simultaneous sensory evaluation of different storage times for a particular sample.

Design A. At each of the nine sample withdrawal times sensory evaluations of the six experimental samples were performed three times (replicates). Each time all samples were presented simultaneously in randomized order, with different orders for the different judges. For each attribute, intensity data were entered into a two-way analysis of variance, ANOVA, to study the variables: (1) samples (df = 5), and (2) judges (df = 6-8), and their interaction.

Design B. At each withdrawal time, some samples were put into storage at $-80°C$. Immediately after completion of the $-20°C$ storage experiment, three of the experimental samples (I, IV and V) from four of the storage times (0, 5, 16, and 28 wk) were chosen for experiment B. The 12 samples (3 samples × 4 storage times) were presented to the judges and evaluated as in experiment A. The evaluation was performed three times. For each attribute all data were inserted into a three-way ANOVA with the variables: (1) samples (df = 2), (2) storage time (df = 3), and (3) judges (df = 6). Note that e.g. storage time 16 wk here means 16 wk at $-20°C$ followed by at least 12 wk at $-80°C$, etc.

GC analysis of headspace volatiles

A headspace sampling procedure (von Sydow et al., 1970) was used to concentrate volatiles from the herring homogenates. A 50-g sample of the mince was homogenized with 75 mL activated-carbon-filtered distilled water. A 100-g portion of the homogenate was then weighed into a 500-mL headspace sampling flask, flushed with helium, and equilibrated at 25°C for 30 min. The volatiles in 350 mL headspace gas were collected in a liquid-nitrogen-chilled trap on a concentration/injection system mounted on a Perkin Elmer 900 gas chromatograph. A stainless steel column (0.75 mm × 180 m) coated with SF 96/Igepal, 95/5, was used. The carrier gas was He and the flow rate was 12 mL/min. The injector and the detector temperatures were 100°C and 180°, respectively, and the column had an initial 3-min period at 20°C followed by a 2°C/min increase to 140°C. A Finnigan 4023 GC/MS/DA system was used to identify the volatile compounds.

Peroxide value determination

The fat from 20g mince was extracted with chloroform-methanol as described by Bligh and Dyer (1959) and the peroxide value determined by iodometric titration according to AOAC Method No. 28.023 (AOAC, 1970). Linear regression analysis was used to determine equations describing the increase of the peroxide values during storage. The slopes of the regression equation of different samples were compared according to Davies and Goldsmith (1972).

RESULTS & DISCUSSION

Sensory properties

At all nine withdrawal times, no statistically significant rancidity difference among the six samples was obtained. Accordingly the sensory evaluation did not show any of the additives, AX, AXC, or HGC, to have a significant antioxidative effect on the raw herring.

With the attributes "fresh herring", "salted herring", "sourness", and "total odor intensity" significant differences between the samples were obtained only occasionally, and with no seemingly systematic relation to additions of MRP. They were therefore considered coincidental. Significant "sweetness" differences were obtained, however, showing that the HGC in sample VI added faint sweetness to the odor.

Table 1—Major volatiles in headspace gas by GC/MS/DA analysis from minced herring with no addition of MRP during the storage period of 0 - 28 wk

Mass spectral peak no.[a]	Compound	Concentration range (ng/L)
1	Propane	68 — 6570
2	Propene	25 — 225
3	Ethanal	67 — 590
4	Propanal + Propanone + C_5H_{10}[b]	54 — 1540
6	Pentane	8.6 — 463
7	2-Pentene[b]	115 — 2610
8	2-Pentene[b] + 1,3-Pentadiene	10 — 1080
11	Methylpropanal	0.9 — 50
12	Butanal + Butanone	25 — 90
14-16[c]	1,4-Hexadiene + Hexane + 3-Hexene	13 — 91
20	3-Methylbutanal	1.4 — 54
21	2-Methylbutanal	15 — 23
23	2-Pentanone	4.5 — 23
24	3-Pentanone	4.9 — 71
26	2-Ethylfuran	n.d.[d] — 17
27,28	1-Penten-3-ol + Heptane	23 — 342
33	Hexanal	0.5 — 33
34	Octadiene[b]	0.4 — 132
38	Octene[b]	0.5 — 26
39	Octadiene[b]	0.3 — 529
41	Octadiene[b]	3.4 — 307
43	2-Hexenal	n.d.[4] — 13

[a] Original mass spectral data are available from SIK.
[b] One of the isomers.
[c] These peaks were not always resolved.
[d] n.d. = not detected.

An increase in the intensity of "rancid herring" with respect to storage time was observed for all samples. Using Design B this increase was shown to be significant.

Formation of volatile compounds

Chromatograms of headspace volatiles of each treatment of minced herring, stored for 0 - 28 wk, showed that many compounds increased with storage time. Table 1 lists the major compounds identified by GC/MS/DA and their concentration in the control. About 50 compounds were identified and the major classes were: saturated and unsaturated hydrocarbons, straight-chain aldehydes, branched-chain aldehydes, ketones, and alcohols. The concentration ranges in the samples with added MRP were similar to those in the control. Several of the compounds occurred in peaks that were not completely resolved. The proportions of different compounds in unresolved peaks were obtained by analyzing their relative contributions to mass spectra. Hydrocarbons which may be formed via degradation of fatty acids (Grosch, 1982) showed the largest increase during storage. Straight-chain aldehydes and unsaturated alcohols such as 1-penten-3-ol are formed via lipid oxidation. The branched-chain aldehydes, i.e. methylpropanal and 2- and 3-methylbutanal, are formed via Strecker degradation of amino acids.

Formation of peroxides

The changes in peroxide values with time for the control and the five samples with additions of MRP were found to increase considerably. After 28 wk, peroxide values of approximately 30 meq/kg were reached in all samples. There were no statistically significant differences among samples with different additions.

DISCUSSION

THE ADDITION of MRP to the fish mince was expected to retard the formation of volatile oxidation products;

Differential Scanning Calorimetry of Fish Muscle: The Effect of Processing and Species Variation

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ANTHONY D. MATTHEWS, and EVELYN M. ANDERSON

ABSTRACT

Differential Scanning Calorimetry (DSC) has been used to study the thermal properties of fish muscle proteins and to measure the extent of their denaturation under various processing conditions. Fish myosin was susceptible to denaturation by frozen storage and dehydration. Denaturation of certain fish proteins was partially reversible. Although fish myosin was very unstable, its thermal stability was found to increase in species adapted to higher environmental temperatures.

INTRODUCTION

DIFFERENTIAL SCANNING CALORIMETRY (DSC) is a very useful means of studying the thermal properties of muscle proteins (Wright et al., 1977) and of protein denaturation (Arntfield and Murray, 1981). The behavior of the proteins can be studied in situ, and there is no need for solubilization of the sample. The technique has allowed the thermally induced transitions of rabbit and beef actin, myosin and sacroplasmic proteins to be studied as a function of pH and ionic strength (Wright et al., 1977; Stabursvik et al., 1980). The effects of process conditions on beef and vegetable protein denaturation have also been investigated (Quinn et al., 1980: Arntfield and Murray, 1981). However, apart from a study which included a thermogram of cod muscle (Martens and Vold, 1976), there has been little DSC analysis of muscle proteins from marine sources. This seems a peculiar omission for two main reasons: (1) Fish muscle proteins are much more unstable than mammalian to physical processes such as freezing and frozen storage (Shepherd, 1960). DSC would help to establish whether this instability exists during other food manufacturing processes. (2) The fact that fish species live in polar as well as tropical waters ($-2°C$ to $28°C$) without any control over their body temperature affords an opportunity of establishing whether the thermal denaturation characteristics of fish muscle proteins are dependent on the environmental conditions. Some previous studies have measured the myosin ATPase activity as a function of environmental temperature (Johnston et al., 1973). Parallel changes in the thermally induced unfolding of the myofibrillar proteins have not been examined.

The work described in this paper therefore attempts to characterize qualitatively, using DSC: (1) The extent of denaturation incurred by the muscle proteins of cod during freezing, frozen storage and drying; and of herring during marinading. (2) The thermal properties of the muscle proteins from fish species adapted to different environmental temperatures.

MATERIALS & METHODS

COD (*Gadus morhua*) were obtained live from a local fisherman, and kept in an aquarium until needed. Herring (*Clupea harengus*) and squid (*Loligo forbesi*) were obtained fresh (2 – 3 days old) from the Aberdeen Fish Market. Jumbo Tiger Prawns (*Penaeus monodon*) were donated by colleagues involved in Tropical Aquaculture studies. Myofibrils isolated from Arctic Char (*Salvenlinus alpinus*), *Notothenia neglecta*, and *Tilapia mossambica* by the method of Perry and Gray (1956) were obtained from Dr. I. Johnson, St. Andrews Univ., Fife, Scotland. The environmental temperatures of the fish which were subjected to the comparative study are summarized in Table 1.

Protein purification

Muscle proteins of pre-rigor cod were isolated for DSC studies as follows: An acetone *actin* powder was prepared according to the procedure of Tsao and Bailey (1953). Actin was extracted from the acetone powder by the method of Seki et al., (1973). *Myofibrils* were prepared by the method of Goodno and Swenson (1975). *Sarcoplasmic proteins* were prepared by extracting minced cod muscle with 0.1M KCl, 2 mM $MgCl_2$, 20 mM KH_2PO_4 (pH 7, μ = 0.1) for 45 min. The slurry was strained through gauze and the filtrate centrifuged at 40,000 x g for 90 min at +2°C. The supernatant was dialyzed against the extraction buffer for 24 hr and centrifuged again. The supernatant was concentrated by ultra-filtration and used for DSC analysis. Connective tissue, as a source of *collagen*, was dissected from the myocommata of the larger cod used in the study.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry was performed on a Perkin-Elmer DSC II. The instrument was temperature calibrated using water and benzil. Samples weighing 10 – 15 mg with an accuracy of ± 0.01 mg were sealed in Perkin-Elmer volatile sample pans. Reference pans contained an equal weight of water. The samples were scanned at a heating rate of 10° K/min over the range 274 – 370°K at an instrument sensitivity of 0.1 or 0.2 mcal/sec. The machine constant used to calculate enthalpies (ΔH) was obtained from thermograms of indium calibration standards. The apparent transition heat, ΔH_{app}, was determined from the peak area and expressed in mcal/mg dry weight. It was most reproducible to record peak transition temperatures as peak maxima (Tm).

Process conditions

Three different processes which are well established in the food industry were used.

Freezing and frozen storage. Fresh post-rigor cod fillets were minced on a Baader 694 deboning machine, blast frozen at $-40°C$ and stored at $-10°C$. Samples were removed for DSC analyses after 0, 2, 6 and 10 wk.

Table 1—Average temperature of the environment of various fish species

Species	Environmental temperature °C
Gadus morhua	0 to 6
Clupea harengus	4 to 8
Loligo forbesi	2 to 6
Penaeus monodon	25 to 30
Salvelinus alpinus	0 to 4
Notothenia neglecta	−2 to 0
Tilapia mossambica	25

Salting/Marinading (high ionic strength, low pH). Herring fillets were soaked in stirred solutions of (1) 14% w/w salt, (2) 7% w/w acetic acid and (3) 14% w/w salt and 7% w/w acid at a fish/solution ration 1:1 for 4 days to allow equlibration to occur. (The herring used in this study had a 70% water content. Assuming all this water is free to equilibrate, then the final levels of salt and acid in the aqueous phase are approximately 8% (1.5M) and 4% respectively). However, as well as determining the effects of acid and salt as "denaturants" of herring muscle proteins, the reversibility of these changes was assessed. Samples of fish were therefore dialyzed against running water for 2 days.

Drying. Skinned cold fillets were cut into 1.5 cm^3 dice, spread on trays and dried in a Torry kiln at 4 different temperatures: 30°, 45°, 60° and 90°C. The corresponding wet bulb temperatures were 28°, 39°, 47° and 60°C respectively. Samples were taken at intervals during the course of drying. Duplicate samples were allowed to rehydrate overnight in an excess of cold water (2°C). In this way it was possible to determine not only the effect of reduced moisture content on muscle protein denaturation, but also whether the effects were reversible.

RESULTS & DISCUSSION

THE DSC RESULTS were recorded as "thermograms," which are plots of the differential heat input (mcals per second) against temperature. In each DSC analysis the sample and reference were heated at a constant rate (10°K/min) from 274°K to 370°K. As the temperature rose, heat-induced endothermic protein unfolding occurred in the sample. The unfolding was registered by the DSC as the additional energy required by the protein sample to match the linear temperature increase of the reference. The energy was plotted as peaks on the thermogram. Baselines were fitted to each curve to allow calculation of areas and enthalpy changes. Changes in protein structure during DSC analysis are sometimes referred to as "transition" changes.

Characterization of thermal transitions of cod muscle

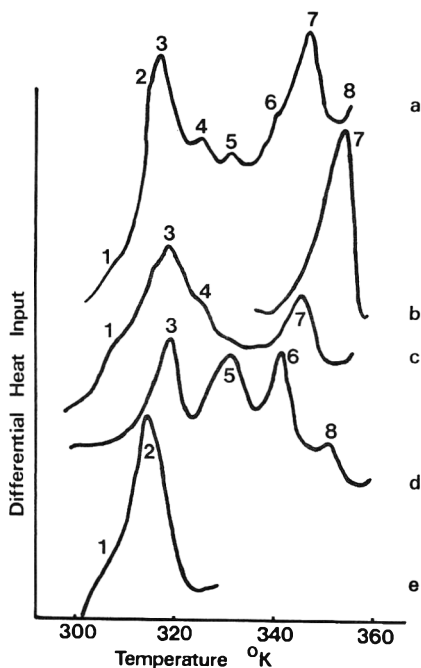A comparison of DSC thermograms of various purified cod muscle proteins is shown in Fig. 1. It is apparent that

cod muscle has the same basic DSC profile as rabbit muscle, made up from a pattern of myosin, sarcoplasmic proteins and actin (Wright et al., 1977). The main differences were that the major myosin transition in cod occurred at about 10°K below that in rabbit, and more peaks were observed in isolated sarcoplasmic proteins from cod than from rabbit. On the basis of our results on isolated cod proteins and assuming that cod DSC profiles follow a similar pattern to those of rabbit, the peaks on the thermogram for whole cod muscle (a) were labelled 1 to 8 and assigned as follows: (1) composite of first myosin transition and first collagen transition; (2) the second (major) collagen transition; (3) composite of the second (major) myosin transition and the first sarcoplasmic transition; (4) third myosin transition; (5) second sarcoplasmic transition; (6) third sarcoplasmic transition; (7) actin transition; (8) a fourth sarcoplasmic transition is sometimes present. All the proteins were at pH 7.0 and ionic strength between 0.5 and 1.0, except the purified actin, where the ionic strength was 0.05. The transition of purified actin has a slightly higher Tm as a result.

Effect of freezing and frozen storage

Freezing followed by immediate thawing has little effect on the characteristic thermal transitions of cod muscle (Fig. 2a). However, after 2 wk at −10°C (Fig. 2b) it is apparent that myosin has undergone some partial denatuation. The thermograms for the 6 and 10 wk storage period (Fig. 2c and 2d) indicate that (1) there is little subsequent effect on the myosin transition after 2 weeks at −10°C (as measured by DSC) and (2) actin, collagen, and sarcoplasmic proteins are largely unaffected by frozen storage.

Effects of high ionic strength and low pH

The thermogram of untreated herring muscle is shown in Fig. 3. After treatment with 14% salt solution, the transition temperatures were lowered by 5 – 10°K, and the peak

Fig. 1–DSC thermograms of cod proteins: (a) Whole cod muscle; (b) Actin; (c) Myofibrils; (d) Sarcoplasmic proteins; (e) Collagen. The peaks are numbered for discussion in the text.
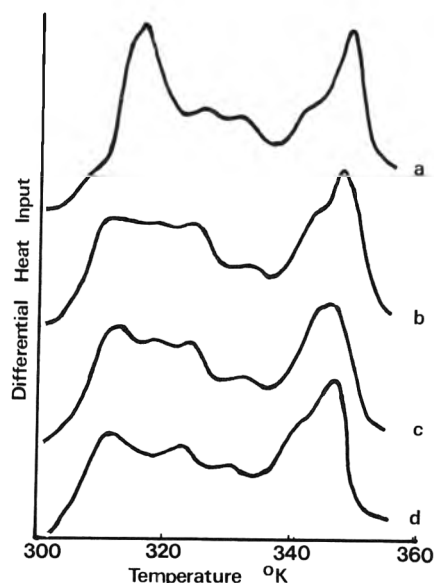
Fig. 2–DSC thermograms of cod muscle during 10 wk frozen storage at −10°C: (a) Control (frozen and thawed immediately); (b) 2 wk; (c) 6 wk; (d) 10 wk.
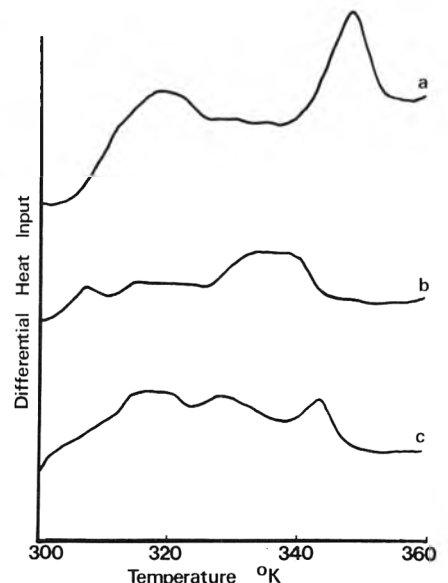
Fig. 3–DSC thermograms of herring muscle: (a) Control, untreated; (b) Soaked in 14% salt for 3 hr; (c) Dialyzed against running water for 24 hr.

areas decreased (Fig. 3b). Dialysis of identically treated samples resulted in only *partial* recovery of the peak areas and restoration of the original transition temperatures. This supports the results of an earlier study by Stabursvik and Martens (1980) who showed that salt depressed transition temperatures in beef muscle suggesting that the muscle protein structure was destabilized.

At the equilibrated pH level of acid treated fish (pH 4.0), the myosin and actin transitions in herring muscle were almost completely lost (Fig. 4a), but part of the original thermogram was recovered after sample dialysis (Fig. 4b). When herring was soaked in 14% salt and 7% acid, only one low broad peak with Tm = 318°K, was seen (Fig. 5). After sample dialysis, the resulting thermogram indicated that actin had recovered almost completely, but myosin less so. The major observation from this part of the study is that myosin and actin are *not* irreversibly denatured after exposure to high ionic strength (∼1.5M) and low pH (4.0), even when these "harsh" conditions are combined. Certainly, myosin does not "renature" fully, but actin exhi-

bits a significant degree of recovery of its native conformation.

Effect of drying

DSC thermograms of cod muscle dried for 2 hr at four temperatures are shown in Fig. 6. Thermal transitions were lost completely if the wet bulb temperature during drying exceeded the natural transition temperature. As the moisture content of the muscle decreased, transition temperatures increased (Fig. 7). The relationship between the transition temperature for actin and the muscle moisture content is shown in Fig. 8, for drying at 45°C. No thermal transitions remained after overnight drying at 30° or 45°C, after 4 hr at 60°C or 1 hr at 90°C (Table 2). The corresponding wet bulb temperatures are given in the methods section. There are two possible explanations for this observation: (1) irreversible heat denaturation had occurred during drying, or (2) there was no water medium to allow thermal unfolding (Hagerdal and Martens, 1976).
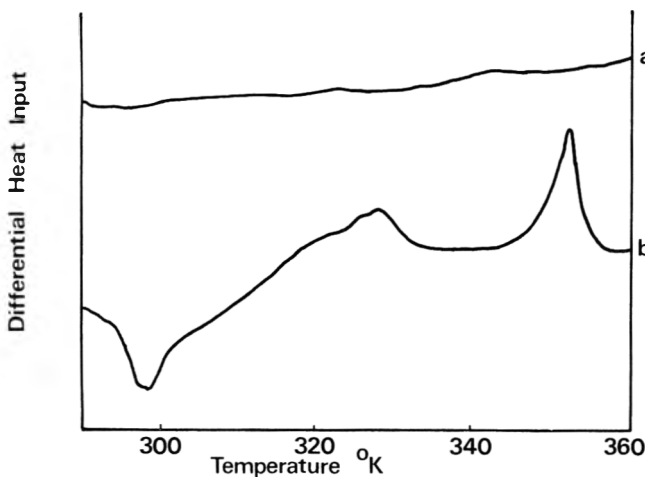

Fig. 4—DSC thermograms of herring muscle: (a) Soaked in 7% acid for 2 min; (b) Dialyzed against running water for 24 hr.
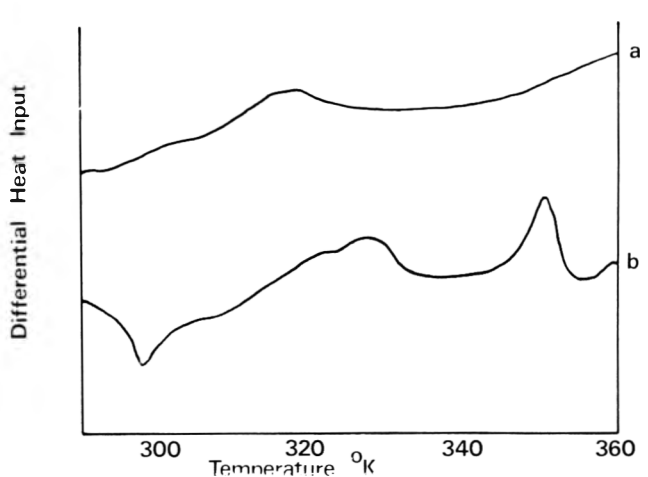

Fig. 5—DSC thermogram of herring muscle: (a) Soaked in 14% salt and 7% acid for 2 min; (b) Dialyzed against running water for 23 hr.
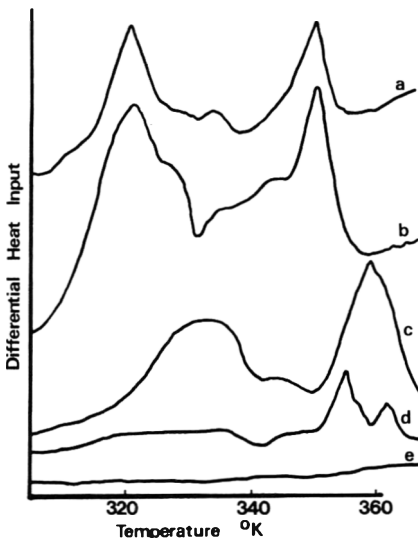

Fig. 6—DSC thermograms of cod muscle dried for 2 hr in the Torry kiln: (a) raw cod; (b) dried at 30°C; (c) 45°C; (d) 60°C, (e) 90°C.
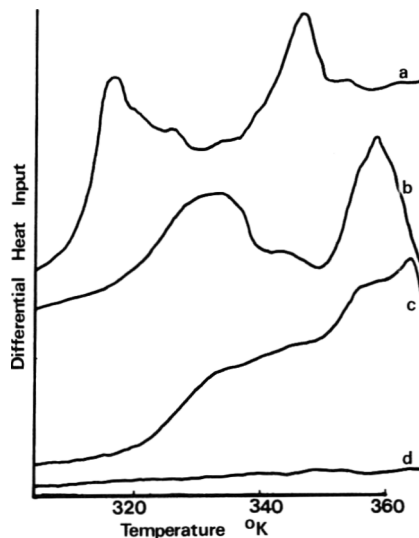

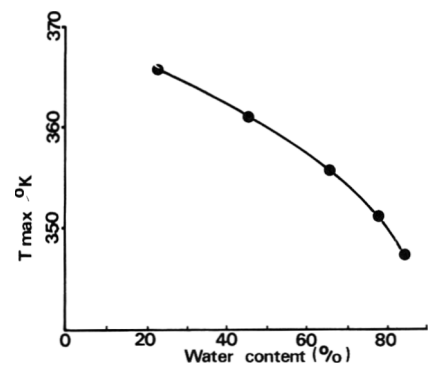Fig. 7—DSC thermograms of cod muscle dried in the Torry kiln at 45°C: (a) 0 hr; (b) 2 hr; (c) 4 hr, (d) 22 hr.

Fig. 8—Transition temperature (Tm) of actin as a function of water content of cod muscle dried at 45°C.

The reversibility of the apparent denaturation caused by drying is shown in Fig. 9. The extent to which the original thermograms were recovered after rehydration depended on the wet bulb drying temperature (Table 3). Actin recovery was more complete at the lower drying temperatures. There was considerable irreversible myosin denaturation irrespective of drying temperature, again highlighting the relative instability of fish myosin compared to fish actin. In their study of myoglobin (Hagerdal and Martens, 1976) suggested that proteins became more stable to heat as the moisture content fell because inter- and intra-molecular electrostatic interactions and hydrogen bonds became established in sites earlier occupied by water. This could well explain our own observations. For myosin however, loss of water may initiate the type of irreversible aggregation reactions which are presumed to occur during frozen storage.

Species differences

Typical DSC thermograms of raw muscle from cod, herring, squid and jumbo tiger prawn are shown in Fig. 10. The profiles displayed a similar pattern of myosin and actin peaks. However, aquid and jumbo tiger prawn had more diffuse actin peaks than cod or herring and jumbo tiger prawn had two peaks near the actin position. Cod, herring and squid are North Atlantic species adapted to similar environmental temperatures, $0°$ to $8°C$ (Table 1). Jumbo tiger prawns are a tropical species, adapted to temperatures of approximately $25 - 30°C$. The DSC shows that the jumbo tiger prawn muscle proteins are more thermostable than the three North Atlantic species. The first peak on the thermogram of the prawn muscle is approximately $10°C$ higher than the other species.

DSC thermograms of myofibrils from *Notothenia*, Artic char and *Tilapia* are shown in Fig. 11. *Notothenia neglecta* inhabits Antarctic waters ($0°$ to $-2°C$), Arctic char is a cold water species ($0 - 4°C$) and *Tilapia* is found in the trpics ($25 - 30°C$). The profiles showed major myosin peaks followed by a small actin peak at approximately $340°K$ and demonstrated again that proteins of warm water species were more thermostable than those of cold water species. The myosin transition was at a higher temperature in *Tilapia* than in the cold water species. These results are good evidence to support the general statement that myosin is more thermostable in species adapted to warm water.

In summary, using DSC as a tool for measuring protein denaturation, it has been shown that fish muscle myosin is very unstable and easily irreversibly denatured during handling and processing. Perhaps because of this intrinsic instability, the conformation of the molecule has evolved so that its thermal denaturation temperature is higher in warm water fish. Actin on the other hand is much more stable and its "denaturation", unless by heat, is largely reversible.

Table 2—Average apparent transition heat of cod muscle dried at 30°C, 45°C, 60°C and 90°C

Time of drying	Temperature of drying			
	30°C	45°C	60°C	90°C
Raw Cod	3.88[a]	5.49	3.36	2.55
30 min	—	—	—	0.94
1 hr	3.68	2.98	1.95	0.24
2 hr	4.52	3.58	0.690	0.00
4 hr	3.82	1.90	0.127	—
6 hr	3.43	0.46	0.00	—
22 hr	0.00	0.00	—	—

[a] Units are mcals per mg dry weight

Table 3—Recovery of apparent transition heat in cod muscle rehydrated from dried pieces showing no thermal transitions

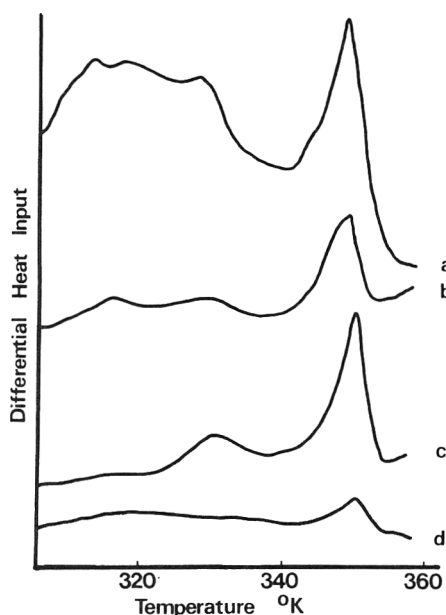| Drying temp (°C)	Wet bulb temp (°C)	Average Δ Happ. (mcal/mg)
30	28	2.25
45	39	1.39
60	47	0.51
90	60	0.14

Fig. 9—DSC thermogram of cod muscle dried completely at the indicated temperatures and rehydrated in water for 24 hr: (a) 30°C; (b) 45°C; (c) 60°C; (d) 90°C.
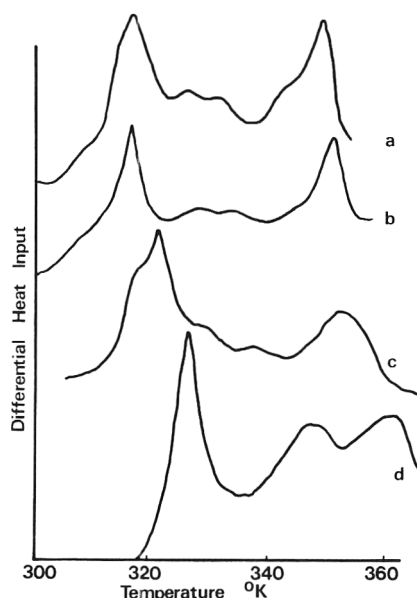

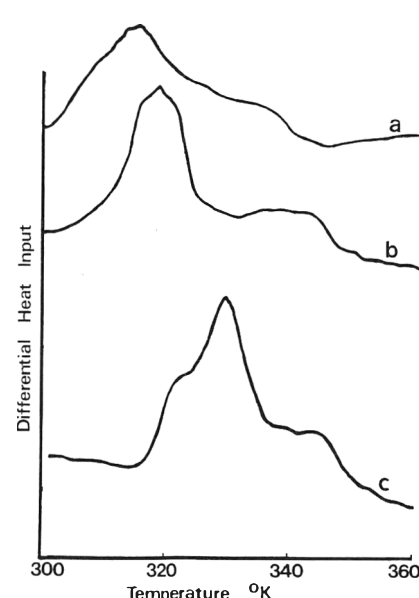Fig. 10—DSC thermograms of fish muscle: (a) Cod; (b) Herring; (c) Squid; (d) Jumbo tiger prawns.

Fig. 11—DSC thermogram of myofibrils: (a) Notothenia neglecta (Antarctic fish); (b) Salvelinus alpinus (Arctic char); (c) Tilapia sp. (Tropical fish).

Heat-Induced Aggregation and Denaturation of Egg White Proteins in Acid Media

KENJI WATANABE, TSUKASA MATSUDA, and RYO NAKAMURA

─────────── ABSTRACT ───────────

Heat-induced aggregation and denaturation of egg white proteins adjusted to pH 5.5, 4.5, 3.5 and 2.5 were investigated by vertical flat-sheet polyacrylamide gel electrophoretic and differential scanning calorimetric methods. The fractional and step-wise aggregation of egg white proteins was caused by heating. As the acidity was increased from pH 5.5 to 2.5, ovotransferrin, ovomacroglobulin, globulin G3A, globulins A1 and A2, and ovalbumin became much more unstable to heat. However, ovomucoid and ovoinhibitor did not aggregate in the acidified egg white under heat treatments of 3 min at 90°C or 20 min at 74°C. The heat-induced aggregation of flavoprotein was slightly greater at pH 4.5 and 3.5 than at pH 5.5 and 2.5.

INTRODUCTION

THE SENSITIVITY of egg white proteins to heat denaturation and heat aggregation has been a subject of interest (Chang et al., 1970; Cunningham and Lineweaver, 1965; Nakamura and Matsuda, 1983; Seideman et al., 1963), because the ability of egg white to heat-coagulate is the basis for its use in a wide variety of food products. In the previous study (Matsuda et al., 1981), a vertical flat-sheet polyacrylamide gel electrophoretic method was used to investigate the heat-induced aggregation of proteins. Results showed the fractional and step-wise aggregation of egg white proteins caused by heating.

Although heat-induced changes of egg white in the acidic pH range have been investigated in single protein systems in the presence and absence of detergents (Egelandsdal, 1980; Ericsson et al., 1983; Foster and Rhees, 1952; Hegg, 1979; Hegg et al., 1978, 1979; Hegg and Löfquist, 1974; Shimada and Matsushima, 1980), information is still limited concerning the egg white system (Cunningham and Cotterill, 1964).

The aim of the present study was to obtain a better understanding of the heat denaturation and heat aggregation of egg white in acid media. Such analyses might be helpful in developing new uses for egg white.

MATERIALS & METHODS

Egg white

Fresh egg white was prepared from 1- to 2-day-old eggs produced by a strain of White Leghorn Layers. The egg white was carefully homogenized without foaming, and the pH of the homogenized egg white was adjusted to 5.5, 4.5, 3.5 and 2.5 with 1N HCl. The pH adjusted egg white samples were used without centrifugation treatment.

Heat treatment

Heat treatment of egg white (1 mL) was carried out as described in the previous report (Matsuda et al., 1981). All samples after heat treatment and cooling were diluted with 1 mL 0.01M NaCl, and centrifuged at 1600 x g for 25 min in order to remove aggregated proteins. Five μL of the supernatant was applied to the electrophoresis according to the method described previously (Matsuda et al., 1981).

Determination of protein content

Protein content of the supernatant was measured by the method of Lowry et al. (1961) using bovine serum albumin as a standard, and expressed as a percentage for the amount of protein in the supernatant of the same treated sample at pH 5.5 without heating.

Differential scanning calorimetry (DSC) measurement

DSC curves were recorded on a Daini Seikosha model SSC/560 thermal analyzer with a heating rate of 1.5°C/min in the temperature range 25 – 100°C. Samples (50 μL) of the pH adjusted egg white and standard protein solutions (each 6% in 0.01M NaCl) were pressure-sealed in silver pans. An identical sealed pan filled with 53 μL distilled water, adjusted to the same pH as sample, was used as a reference. The denaturation temperature was defined as the temperature of the DSC peak maximum.

RESULTS

FIG. 1 SHOWS electrophoretic patterns of egg white (pH 5.5, 4.5, 3.5 and 2.5) which had been heat-treated for 3 min at various temperatures with 4° intervals from 50°C to 90°C. The assignment of the proteins separated during
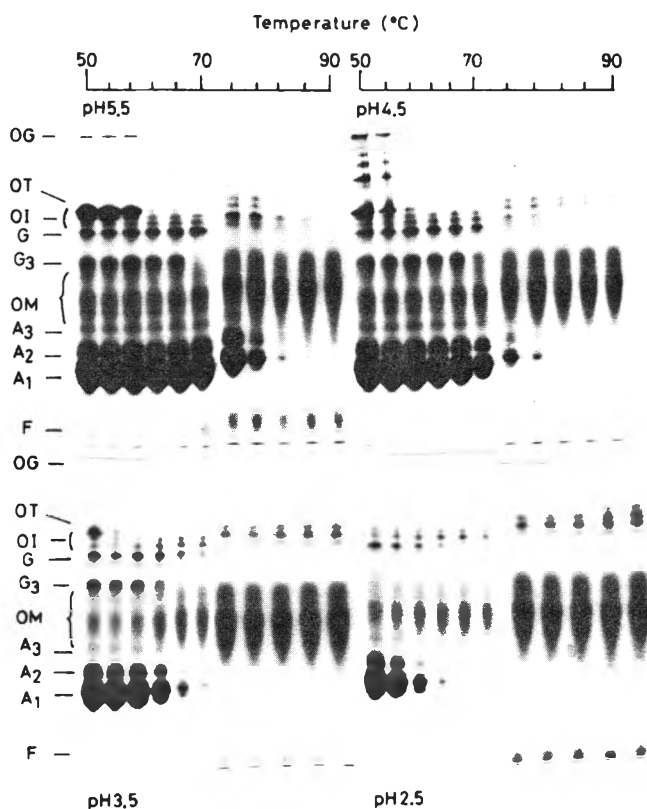

Fig. 1—Electrophoretic patterns of egg white (pH 5.5, 4.5, 3.5 and 2.5) heated for 3 min at various temperatures with 4°C intervals from 50 – 90°C. OG = ovomacroglobulin; OT = ovotransferrin; OI = ovoinhibitor; G = globulins A1 and A2; G3 = globulin G3A; OM = ovomucoid; A1, A2 and A3 = ovalbumin; F = flavoprotein.

electrophoresis was followed by comparison with the result shown in the previous study (Matsuda et al., 1981). In the addition to the assigned proteins, some soluble aggregates in the sample with pH 4.5 heated at lower temperature could be found between ovomacroglobulin and ovotransferrin. These might be due to the proteins which were denatured and aggregated during the preparation of samples and heat treatments, but could not be removed by centrifugation for the small-sized aggregates.

The electrophoretic patterns in Fig. 1 also indicated that the temperature at which proteins disappeared was different in terms of pH at heating: the aggregation occurred at a lower temperature as the acidity was increased. The approximate order of disappearance of each protein band caused by increasing temperature in each sample was as follows: ovotransferrin, ovomacroglobulin, globulin G3A, globulins A1 and A2, and ovalbumin (Table 1). Ovotransferrin and ovomacroglobulin disappeared in the samples
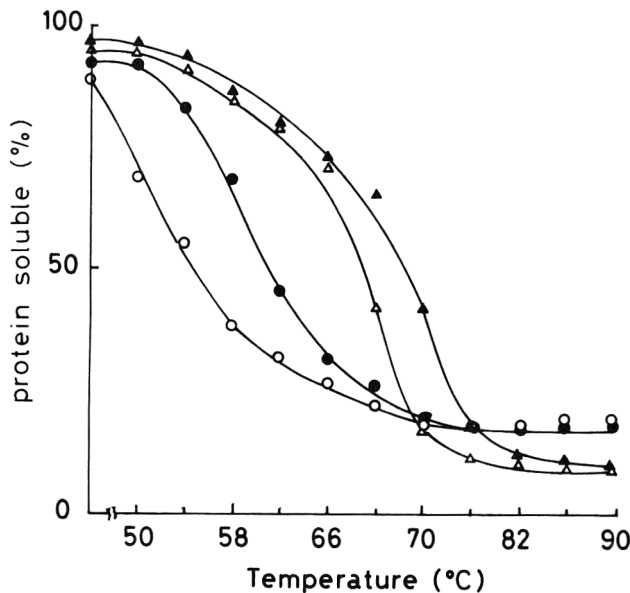
adjusted to pH 3.5 and 2.5 and heated at 50°C, except for the slightly residual ovotransferrin in the sample at pH 3.5 and 50°C. The ovomucoid band remained almost completely even in the pattern of all samples heated at 90°C. The ovoinhibitor band remained in the samples of pH 4.5, 3.5 and 2.5 heated at 90°C, whereas it disappeared at 82°C in the sample heated at pH 5.5. The flavoprotein band, which remained in the samples heated at pH 5.5 and 2.5, became more indistinct at pH 4.5 and 5.5

Fig. 2 shows changes in the soluble proteins in the samples used for the electrophoretic experiments in Fig. 1. A steep change in solubility at lower heating temperatures was observed for the sample heated at pH 2.5. The precipitated protein was successively diminished with increasing pH at heat treatment. However, even heating at pH 2.5 could not result in the disappearance of all of the proteins, and remaining protein content at heating temperatures above 78°C was slightly higher at pH 3.5 and 2.5 than at pH 5.5 and 4.5. Such remaining proteins could be recognized to consist mainly of ovomucoid and ovoinhibitor, juding from electrophoretic patterns in Fig. 1. Such a slight difference in the precipitated amount at higher heating temperature could be due to the difference in the stability of the remaining proteins in the acidic media.

The electrophoretograms of samples heated at 58° and 78°C for various periods (0 – 20 min) are shown in Fig. 3 and 4, and Fig. 5 and 6 indicate changes in the protein contents in the supernatant after heat treatments at 58° and 78°C, respectively. At pH 5.5 and 4.5 at 58°C, a slight reduction in protein content could be found during heating for 20 min, depending on the disappearance of ovotransferrin and ovomacroglobulin (Fig. 3 and 5). At pH 3.5 and 2.5 at 58°C, ovotransferrin, ovomacroglobulin, ovalbumin, globulin G3A and globulin A1 and A2 were de-

Fig. 2—Changes in the soluble protein contents in the supernatants (-▲-, pH 5.5; -△-, pH 4.5; -●-, pH 3.5; -○-, pH 2.5) used for electrophoretic experiment in Fig. 1.

Table 1—Disappearance temperatures of egg white protein bands in Fig. 1 by heating

Protein[a]	Adjusted pH			
	pH 5.5	pH 4.5	pH 3.5	pH 2.5
OG	68	58	<50	<50
OT	62	58	54	<50
OI	82	90	>90	>90
G	82	74	66	62
G3	70	70	62	50
OM	>90	>90	>90	>90
AI	82	78	68	62

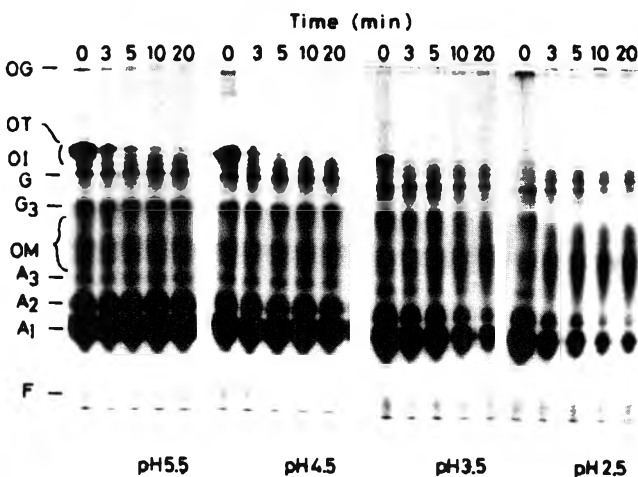[a] See Fig. 1 for definition of protein band abbreviation.

Fig. 3—Electrophoretic patterns of egg white (pH 5.5, 4.5, 3.5 and 2.5) heated at 58°C for various periods of time (0 – 20 min). See Fig. 1 for definitions of protein band abbreviations.
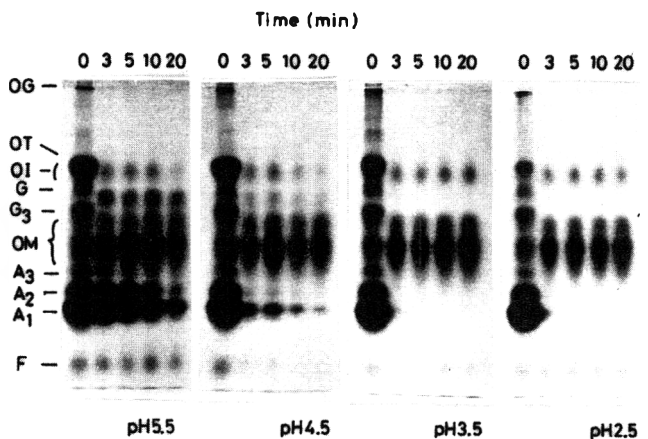

Fig. 4—Electrophoretic patterns of egg white (pH 5.5, 4.5, 3.5 and 2.5) heated at 74°C for various periods of time (0 – 20 min). See Fig. 1 for definitions of protein band abbreviations.

creased with the heating time (Fig. 3). By heating at 78°C, nearly all of the precipitable proteins in egg white was sharply precipitated from heating for 3 min in the samples of pH 4.5, 3.5 and 2.5, and the precipitation of the sample of pH 5.5 gradually occurred for 20 min (Fig. 4 and 6).

Fig. 7 shows DSC thermograms of egg white, of which the pH was adjusted to the acidic side. Some variations in the positions of the peaks in the thermograms could be observed in terms of pH of the sample. The thermograms of the samples of pH 5.5 and 4.5 show two main peaks at 58 – 62°C and 66 – 82°C, at 44 – 56°C and 62 – 78°C, respectively, while those of pH 3.5 and 2.5 indicate one main peak at 56 – 68°C and 52 – 62°C, respectively. As determined in separate experiments with the purified egg white proteins solubilized with 0.01N NaCl solution and adjusted to the desired pH, the ovalbumin denaturation peak was shifted to a lower temperature by lowering the pH from 5.5 to 2.5. The main peaks in each thermogram (denaturation temperatures: pH 5.5 – 76.0°C, pH 4.5 – 70.0°C, pH 3.5 – 62.5°C, pH 2.5 – 56.6°C) corresponded to ovalbumin denaturation. The peaks at lower temperatures (pH 5.5 – 58.0°C and pH 4.5 – 48.5°C) also corresponded to ovotransferrin and the thermogram peak of ovotransferrin at pH 3.5 and 2.5 did not appear because the denaturation of the protein already occurred when subjected to the pH adjustment. The other egg white proteins did not appear as separate peaks on the egg white thermogram because they were minor proteins and were concealed by the large ovalbumin peak, as described by Donovan et al. (1975). Thus, the remarkable pH sensitivity for denaturation of the two main constituents of egg white, ovalbumin and ovotransferrin, could be demonstrated.

DICSUSSION

IT IS TRUE that pH of egg white has a profound effect on heat coagulation of the component proteins. However, the results are greatly influenced by the criteria used for determining coagulation. For example, egg white is more stable to heat at pH 6.5 than at pH 8.5 as determined by gelation, but lowering of pH reduces the coagulation temperature based on optical density measurements (Vadehra and Nath, 1973). Egg white was inclined to gel at acidic sides (pH 3.5 and 2.5) and coagulate near the isoelectric point (pH 5.5 and 4.5) (Egelandsdal, 1980). Flat-sheet polyacrylamide gel electrophoresis proved to be an effective technique for investigating the heat-induced aggregation of individual proteins in egg white using the soluble proteins after removing centrifuged precipitate, either gel or coagulate. In the present study, as well as the earlier results at

Fig. 5—Changes in the soluble protein contents in the supernatants (-▲-, pH 5.5; -△-, pH 4.5; -●-, pH 3.5; -○-, pH 2.5) used for electrophoretic experiment in Fig. 3.
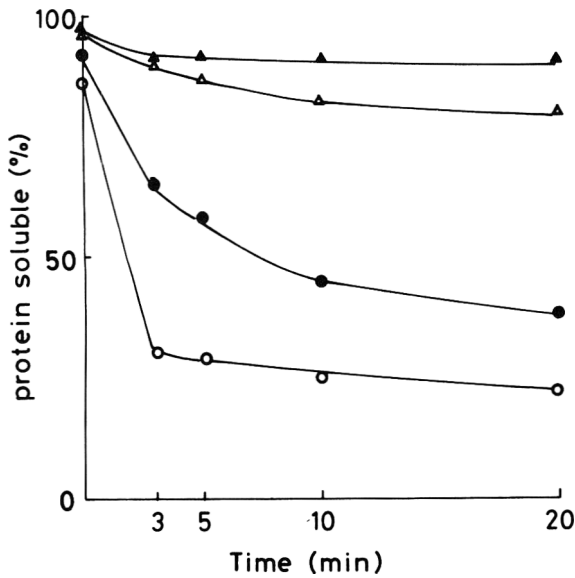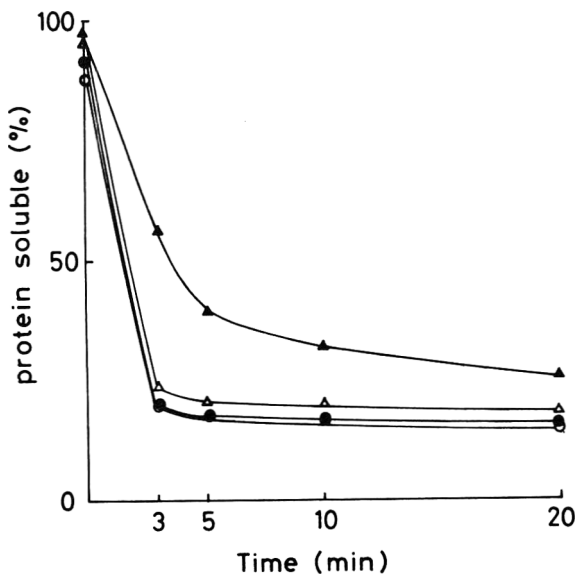

Fig. 6—Changes in the soluble protein contents in the supernatants (-▲-, pH 5.5; -△-, pH 4.5; -●-, pH 3.5; -○-, pH 2.5) used for electrophoretic experiment in Fig. 4.
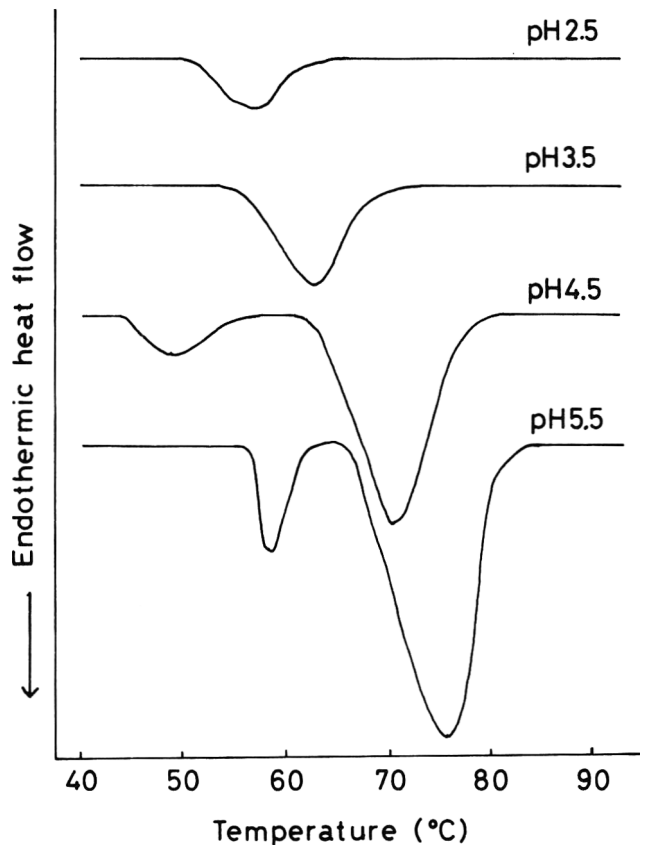

Fig. 7—Differential scanning calorimetric thermograms of egg white (pH 5.5, 4.5, 3.5 and 2.5).

pH 7 and 9 (Matsuda et al., 1981), a heat-induced fractional and step-wise aggregation of egg white proteins at acidic pH region was caused. It has been demonstrated that the aggregation rate of globulin A1 and A2 at 76°C in egg white (pH 7 and 9) was higher than that of ovalbumin, and globulin G3A was unstable under heat treatment at pH 7 and 9. In the acidic pH range the aggregation temperature of globulin A1 and A2 was almost the same as ovalbumin, and that of globulin G3A was lower than ovalbumin.

The high degree of heat denaturation and heat aggregation of ovalbumin near the isoelectric point was entirely expected, because the aggregation temperature of its protein has been reported to be practically constant at a wide pH range but with the absolute minimum at pH 5.5 (Vadehra and Nath, 1973). In this report, ovalbumin in egg white proved to be more unstable on the acidic pH side than in the vininity of the isoelectric point. It has also been reported that at or near pH 2 there was substantially no aggregation of ovalbumin in salt-free solution, but addition of salt to salt-free heat-denatured samples leads to a pronounced turbidity increase. Thus, ovalbumin has a tendency to aggregate by heat on the acidic side depending on the ionic strength. Therefore, the aggregation of ovalbumin presumably differed in terms of the heating condition and the methods for measuring aggregation.

Of the major proteins in egg white on the acidic side, ovotransferrin is the most sensitive to heat and becomes insoluble in egg white upon denaturation. In order to increase the heat stability of ovotransferrin and decrease the damage resulting from a heat treatment of egg white, the stabilization of ovotransferrin with metal ions has been utilized in the food industry. However, an important point is that the metal ovotransferrin complexing is pH-dependent, and the lowest pH on the acidic side that can be used is 6.0 (Wishnia et al., 1953). Ovotransferrin has been previously described to become unstable as the acidity was increased; the denaturation of ovotransferrin is complete in seconds at pH 3.2, whereas the reactions take days at pH 4.2 (Wishnia and Warner, 1961). In this study a relationship was established between the aggregation of ovotransferrin (temperature and time) and the pH of egg white.

Although the aggregation temperature of the ovoinhibitor was much lower than for ovomucoid, it was also indicated to be stable in the acidic pH range.

The ovomacroglobulin in acid solution has been reported to dissociate into two subunits of equal weight which have essentially the same fractional ratio (1.6) as the native

molecule (Donovan et al., 1969). From consideration of that result, the loss of ovomacroglobulin in acidic media shown in this report might be due to the dissociation of the molecule.

REFERENCES

Chang, P., Powrie, W.D., and Fennema, O. 1970. Disc gel electrophoresis of proteins in native and heat treated albumen, yolk and centrifuged whole egg. J. Food Sci. 35: 774.
Cunningham, F.E. and Cotterill, O.J. 1964. Factors affecting acid coagulation of the egg white. Poultry Sci. 43: 53.
Cunningham, F.E. and Lineweaver, H. 1965. Stabilization of egg white proteins to pasteurization temperature above 60°C. Food Technol. 19: 1442.
Donovan, J.W., Mapes, C.J., Davis, J.G., and Hamburg, R.D. 1969. Dissociation of chicken egg-white macroglobulin into subunits in acid. Hydrodynamic, spectrophotometric and optical rotatory measurements. Biochemistry 8: 4190.
Donovan, J.W., Mapes, C.J., Davis, J.G., and Garibaldi, J.A. 1975. A differential scanning calorimetric study of the stability of egg white to heat denaturation. J. Sci. Fd. Agric. 26: 73.
Egelandsdal, B. 1980. Heat-induced gelling in solutions of ovalbumin. J. Food Sci. 45: 570.
Ericsson, B., Hegg, P.O., and Martensson, K. 1983. Protection of ovalbumin against irreversible heat denaturation by a cationic amphiphele at high concentration. J. Fd. Technol. 18: 11.
Foster, J.F. and Rhees, R.C. 1952. An investigation by light scattering of aggregation in the heat denaturation of ovalbumin. Arch. Biochem. Biophys. 40: 437.
Hegg, P.O. 1979. Precipitation of egg white proteins below their isoelectric points by sodium dodecylsulphate and temperature. Biochem. Biophys. Acta 579: 73.
Hegg, P.O. and Löfquist, B. 1974. The protective effect of small amounts of anionic detergents on the thermal aggregation of crude ovalbumin. J. Food Sci. 39: 1231.
Hegg, P.O., Martens, H., and Löfquist, B. 1978. The protective effect of sodium dodecylsulphate on the thermal precipitation of conalbumin. A study on thermal aggregation and denaturation. J. Sci. Fd. Agric. 29: 245.
Hegg, P.O., Martens, H., and Löfquist, B. 1979. Effect of pH and neutral salts on the formation and quality of thermal aggregates of ovalbumin. A study on thermal aggregation and denaturations. J. Sci. Fd. Agric. 30: 981.
Lowry, O.H., Resebrough, N.J., Farr, A.L., and Randall, R.J. 1961. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265.
Matsuda, T., Watanabe, K., and Sato, Y. 1981. Heat-induced aggregation of egg white proteins as studied by vertical flat-sheet polyacrylamide gel electrophoresis. J. Food Sci. 46: 1829.
Nakamura, R. and Matsuda, T. 1983. A new protein band appearing in the electrophoretic pattern of egg white heated at below 60°C. J. Food Sci. 48: 87.
Seideman, W.E., Cotterill, O.J. and Funk, E.M. 1963. Factors affecting heat coagulation of egg white. Poultry Sci. 43: 406.
Shimada, K. and Matsushita, S. 1980. Thermal coagulation of egg albumin. J. Agric. Food Chem. 28: 409.
Vadehra, D.V. and Nath, K.R. 1973. Eggs as a source of protein. CRC. Crit. Rev. Food Tech. 4: 193.
Wishnia, A., Weber, I., and Warner, R.C. 1953. The hydrogen-ion equilibria of conalbumin. J. Am. Chem. Soc. 83: 2071.
Wishnia, A. and Warner, R.C. 1961. The kinetics of denaturation of conalbumin. J. Am. Chem. Soc. 83: 2065.
Ms received 8/8/84; revised 10/19/84; accepted 10/27/84.

REFERENCES

Arntfield, S.D. and Murray, E.D. 1981. The influence of processing parameters on food protein functionality. 1. Diffential scanning calorimetry as an indicator of protein denaturation. Can. Inst. Food Sci. Technol. J. 14: 289.
Goodno, C.D. and Swenson, C.A. 1975. Thermal transitions of myosin and its helical fragments. 1. Shifts in proton equilibria accompanying unfolding. Biochemistry 14: 867.
Hagerdal, B. and Martens, H. 1976. The influence of water content on the stability of myoglobin to heat treatment. J. Food Sci. 41: 933.
Johnston, I., Frearson, T., and Goldspink, G. 1973. The effects of environmental temperature on the properties of adenosinic triphosphatase from various species of fish. Biochem. J. 133: 733.
Martens, H., and Vold, E. 1976. DSC studies of muscle tissue protein denaturation. Proceedings of European Meeting of Meat Research Workers. 22 J9 1 - 6.
Perry, S.V. and Grey, T.C. 1956. A study of the effects of substrate concentration and certain relaxing factors on the magnesium activated myofibrillar adenosine triphosphatase. Biochem. J. 64: 184.

Quinn, J.R., Raymond, D.P., and Harwalker, V.R. 1980. Differential scanning calorimetry of meat proteins as affected by processing treatment. J. Food Sci. 45: 1146.
Seki, N., Kitao, M., Konno, K., and Arai, K. 1973. On the purification of actin from fish muscle. Bull. Jap. Soc. Sci. Fish. 39: 1211.
Shepherd, A'D. 1960. In "Conference on Frozen Food Quality." U.S. Dept. Agric., ARS-74-21.
Stabursvik, E. and Martens, H. 1980. Thermal denaturation of proteins in post rigor muscle tissue as studied by differential scanning calorimetry. J. Sci. Foof Agric. 31; 1034.
Tsao, T.C. and Bailey, K. 1953. The extraction, purification and some chemical properties of actin. Biochem. Biophys. Acta 11: 102.
Wright, D.J., Leach, I.B., and Wilding, P. 1977. Differential scanning calorimetry studies of muscle and its constituent proteins. J. Sci. Food Agric. 28: 557.
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Degree of Difference Test Method in Sensory Evaluation of Heterogeneous Product Types

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ABSTRACT

A degree of difference test and its statistical analysis were developed to replace the traditional triangle test for products which vary during production. Several heterogeneous food products and computer simulated data were used in this study. The result shows that the use of the triangle test with a highly variable product results in a high frequency of false statistically significant results being declared. A degree of difference test which measures and tests perceived differences in taste due to other than product variability via the analysis of variance approach is therefore recommended for dealing with heterogeneous products.

INTRODUCTION

ALTERATIONS in ingredient suppliers or in ingredients themselves change products, and the triangle, duo-trio, and paired difference tests are established methods for determining whether or not this change can be detected through the taste of the product. During the course of normal plant production, samples of product from different batches made day to day, or even within a 1-day period, may exhibit a considerable amount of variation. Color, viscosity, and taste variations are apparently due to no real explanation other than time of day and environmental conditions under which the product was made. A certain amount of variation exists within the production cycle of any product, food or nonfood, but the heterogeneous nature of cooking sauces and canned meats makes them even more susceptible to production variability.

This batch to batch and even unit to unit variation is inherent in the product, yet it is possible that it alone can account for significant taste differences in discrimination tests. An alternative method is needed which takes into account product variability when determining the effects of formulation changes on the taste of a product.

In order to determine the most effective test method for evaluating heterogeneous products, the triangle test and the degree of difference test were investigated.

MATERIALS & METHODS

Sensory procedures

Triangle tests with 32 panelists were conducted on deviled hams, chili, vienna sausage, sloppy joe, pizza, and beef stew. Products were prepared according to package directions; products typically eaten at room temperature were served at room temperature. Products were served in disposable containers. The panelists received a small portion of product (from 1/2 of a vienna sausage to 2 tablespoons of the sauce type products). Ordinary room lighting was used throughout the test. Using the same panelists that participated in the triangle tests, a degree of difference test was conducted on the same products using the questionnaire given in Table 1. In the degree of difference test, four samples denoted by X, X_1, X_2, and Y are prepared. In this notation, X is the reference sample, X_1 is a sample from the same batch as X, X_2 is from a different batch

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than X, and Y is the test sample. The task of the panelists is to determine the degree of difference between the reference sample (X) and the three unknown samples (X_1, X_2, Y). Panelists with extensive experience in tasting the product types studied were used. The triangle and the degree of difference tests were performed on the same work day for a particular product to eliminate possible bias due to a time factor.

Statistical procedures

The degree of difference test design consists of three alike or identical samples X, X_1, and X_2, and a test sample Y. Let the sensations evoked by these four samples be also denoted respectively by X, X_1, X_2, and Y. The three sensations X, X_1, and X_2 are assumed to have the same distribution with mean zero and variance σ^2, while the sensation distribution of Y has mean μ and the common variance σ^2. Since X, X_1, and X_2 are assumed to have the same distribution, the comparison between X_1 and X_2 will provide a measure of pure error. In our definition, pure error will include variations due to product heterogeneity and due to panelists.

The null hypothesis in the degree of difference test is H_0: $\mu = 0$, against the alternative H_a: $\mu > 0$. In our experiment with N panelists each panelist provides an observation on X_1, X_2, and Y where each observation is measured on degree of closeness of X_1, X_2, and Y from the reference sample X. Let X_{1j}, X_{2j}, and Y_j denote the scores of samples X_1, X_2, and Y respectively given by the jth panelist. Then $\bar{X}_1 = \Sigma X_{1j}/N$, $\bar{X}_2 = \Sigma X_{2j}/N$, and $\bar{Y} = \Sigma Y_j/N$, j = 1, . . . , N. To test the null hypothesis, we compute the difference $Y - [(\bar{X}_1 + \bar{X}_2)/2]$ and the statistical significance of the difference is tested by the F ratio in the analysis of variance as shown in Table

Table 1—Degree of difference test questionnaire where 0 = no difference and 5 = extremely large difference.

Name ———————————————— Date ————

Product ———————————————— Set no. ————

Sample X is a reference sample. The coded samples *may* or *may not* be different from Sample X. You are looking for *overall flavor* differences only.

Taste Sample X first, then taste each of the coded samples, from left to right. Compare the coded samples against the X sample when making your judgment of degree of overall flavor difference. Make your judgment on *overall flavor differences only* and not appearance or texture differences.

Difference from reference	Sample codes		
No difference			
Very slight difference			
Slight difference			
Moderate difference			
Large difference			
Extremely large difference			
Describe the overall flavor difference, if any: ————			

2. The sums of squares in the analysis of variance table were obtained by standard procedures (Gacula and Singh, 1984), except for the pure error sum of squares (MSp) which was calculated as the sum of the differences in scores between the reference samples X_{1j} and X_{2j} squared divided by 2 (Gacula, 1978).

Computer simulation

In addition to the use of heterogenous products, a computer simulated population under the null hypothesis was generated using SAS (1982) to further compare the triangle and degree of difference tests. A binomial distribution with parameters N and P = 1/3 and corresponding random numbers from 0 to 5 for the degree of difference score populations X_1, X_2, and Y were obtained. The data generated were subjected to the triangle test analysis and the degree of difference test analysis as outlined earlier.

RESULTS & DISCUSSION

THE HOMOGENEITY of the two stimuli in all characteristics but the specified criterion has been emphasized by Amerine et al. (1965) as a prerequisite for the effective use of the triangle test. However, in actual applications the desired homogeneity is generally not met with a number of canned food products. Consequently, the result of a triangle test with these products is suspect. Indeed, it is common to find significant differences from triangle tests due to the inherent variability in batch to batch production of certain canned food products. Therefore, a degree of difference test which takes into consideration production variation in a product was explored as a means to replace the traditional difference tests for these particular products.

Means, standard errors, and significance probabilities for the triangle and degree of difference tests for 13 trials using various products are shown in Table 3. The differences between means $\overline{X}_1$ and $\overline{X}_2$ reflect production variation within trial. For instance, in trial 2 one batch had a mean of 1.06 and another batch had a mean of 2.00. When this variation is greater than the treatment effects being measured we would not be able to establish significant differences between samples. The agreement between the test

methods based on significance probabilities of at least 0.05 level for rejecting the null hypothesis of no difference was poor; that is, only 5 (trials 5, 6, 7, 11, and 13) out of 13 statistical analyses showed the two test methods were in agreement. Note that for trial 7, and other trials as indicated in Table 3, no significant difference was declared when the difference $\overline{Y} - [(\overline{X}_1 + \overline{X}_2)/2]$ was negative. A negative difference usually occurs when the treatment effects are smaller than the variability between batches. With the exception of trial 13, trials 5, 6, 7, and 11 had fairly homogeneous production batches as indicated by the closeness of the mean scores $\overline{X}_1$ and $\overline{X}_2$. In other trials where a large difference existed between $\overline{X}_1$ and $\overline{X}_2$ or the treatment effects were small, such as trials 1, 4, and 12, a significant difference was declared by the triangle test but not by the degree of difference test. Therefore, it is apparent that the disagreement in results between the test methods is due to product and batch heterogeneity.

The several significant results (H_a: $\mu > 0$, accepted) for the triangle test shown in Table 3 can be theoretically explained as follows. According to Ura (1960) and David and Trivedi (1962), a correct response in the triangle test is obtained if the following inequalities hold: $|X_1 - X_2| < |X_1 - Y|$ and $|X_1 - X_2| < |X_2 - Y|$. That is, correct responses are obtained when the absolute difference between the identical samples X_1 and X_2 is less than the absolute difference between X_1 or X_2 and the odd sample Y. It is possible to satisfy the above inequalities and obtain significant differences in the triangle test even if the mean scores for X_1, X_2, and Y are very similar and no significant difference can be quantified by the degree of difference test. For example, hypothetical data that may occur with a heterogeneous product is shown in Table 4.

According to the inequalities, $|1 - 2| < |1 - 5|$, $|1 - 2| < |2 - 5|$, etc., all the panelists should be able to pick the odd sample Y which would lead to a significant difference in the triangle test. However, if we apply the degree of dif-

Table 2—Analysis of variance for the degree of difference test[a]

Source of variance	DF	MS	F ratio
Total	M − 1		
Test vs reference	1	MSt	MSt/MSp
Pure error	N	MSp	
Residual	M − N − 2		

[a] M = total number of observations; MSt = test mean square; MSp = pure error mean square; N = number of panelists.

Table 4—Hypothetical data to illustrate the effect of heterogeneity on triangle test

Panelist	X_1	X_2	Y
1	1	2	5
2	3	2	0
3	5	4	1
4	0	1	3
$\overline{X}$	2.3	2.3	2.3

Table 3—Significance probabilities for the triangle and degree of difference tests obtained from the comparison of heterogeneous food products

Trial product	$\overline{X}_1$	$\overline{X}_2$	$\overline{Y}$	Standard error[a]	Degree of difference test	Triangle test
1. Sloppy Joe	1.31	1.56	1.28	0.22	b	0.009
2. Deviled Ham	1.06	2.00	1.69	0.25	0.608	0.019
3. Deviled Ham	0.97	2.41	1.66	0.26	0.921	0.019
4. Deviled Ham	1.47	2.28	1.34	0.24	b	0.028
5. Chili	0.69	0.94	1.69	0.20	0.001	0.009
6. Chili	1.07	1.48	1.94	0.21	0.019	0.001
7. Pizza	1.31	1.50	1.28	0.24	b	0.240
8. Chili	1.13	2.03	1.66	0.29	0.826	0.003
9. Chili	1.00	1.59	1.63	0.18	0.155	0.019
10. Chili	0.91	1.34	1.97	0.24	0.007	0.243
11. Chili	1.22	1.16	1.91	0.24	0.020	0.002
12. Vienna	1.28	1.31	0.66	0.20	b	0.050
13. Beef Stew	0.52	1.65	1.65	0.24	0.077	0.050

[a] Standard error computed from the pure error mean square.
[b] Not reported because of a negative difference.

Table 5—Significance probabilities for the triangle and degree of difference tests with sample size N = 60

Simulation experiment no.	No. of agreeing judgments/N	Triangle test	Degree of difference test
1	22/60	0.336	0.397
2	17/60	0.831	0.196
3	22/60	0.336	0.854
4	27/60	0.040	0.389
5	22/60	0.336	0.757
6	17/60	0.831	0.562
7	24/60	0.169	0.781
8	22/60	0.336	0.772
9	23/60	0.244	0.149
10	24/60	0.169	0.089
11	23/60	0.244	0.463
12	17/60	0.831	0.854

Table 6—Significance probabilities for the triangle and degree of difference tests with sample size N = 100.

Simulation experiment no.	No. of agreeing judgments/N	Triangle test	Degree of difference test
1	39/100	0.137	0.100
2	39/100	0.137	0.110
3	33/100	0.566	0.846
4	39/100	0.137	0.065
5	33/100	0.566	0.686
6	25/100	0.972	0.308
7	39/100	0.137	0.127
8	27/100	0.929	0.724
9	27/100	0.929	0.356
10	39/100	0.137	0.708
11	40/100	0.100	0.565
12	33/100	0.566	0.929

ference test to this example, we will obtain no significant difference due to all means being equal and the test mean square (MSt) must therefore equal 0. This indicates that when the products to be evaluated vary highly from sample to sample, the triangle test should be used with caution because variability alone can account for significant taste differences in discrimination tests.

In order to provide additional evidence that product variation is the cause of the test method disagreement, computer simulated data were obtained under the null hypothesis (H_0: $P = 1/3$, H_0: $\mu = 0$), that is, there is no significant difference between the odd and the identical samples. The results of applying the triangle and the degree of difference tests to the simulated data are given in Table 5 for N = 60 and in Table 6 for N = 100. In Table 5, the test method did not agree on one experimental comparison (experiment 4); however, with a larger panel size of N = 100, the test methods were in complete agreement in accepting the null hypothesis at the 5% significance level (Table 6). The agreement in the result between the triangle test and the degree of difference test indicates that when the samples to be evaluated are fairly homogeneous, such as the simulated data, both the methods should agree in accepting or rejecting the null hypothesis. On the other hand, when the samples to be evaluated are heterogeneous, we may expect a larger number of false significant results declared by the triangle test than by the degree of difference test. The larger the variability in the data relative to the difference of interest between samples, the smaller the chances that we will obtain significant results by the degree of difference test.

Thus, from the foregoing results, the degree of difference test is more appropriate than the triangle test for determining if a sample of a heterogeneous product is truely different from another sample of that product.

REFERENCES

Amerine, M.A., Pangborn, R.M., and Roessler, E.B. 1965. "Principles of Sensory Evaluation of Food." Academic Press, New York.
David, H.A. and Trivedi, M.C. 1962. Pair, triangle, and duo-trio tests. Technical Report No. 55, Dept. of Statistics, Virginia Polytechnic Institute, Blacksburg, VA.
Gacula, M.C. Jr. 1978. Analysis of incomplete block designs with reference samples in every block. J. Food Sci. 43: 1461.
Gacula, M.C. Jr. and Singh, J. 1984. "Statistical Methods in Food and Consumer Research." Academic Press, Orlando, FL.
SAS Institute. 1982. "SAS User's Guide: Statistics." SAS Institute, Inc., Cary, NC 27511.
Ura, S. 1960. Pair, triangle, and duo-trio tests. Report of Statistical Applications Research, Japanese Union of Scientists and Engineers 7 107.

Color Quality of Blackcurrant Syrups During Storage Evaluated by Hunter L', a', b' Values

GRETE SKREDE

─────────── ABSTRACT ───────────

Free anthocyanin and Hunter a' value deterioration in blackcurrant syrups during storage followed first order reaction kinetics. Half-life of Hunter a' values were 3 - 4 times higher than half-life values of free anthocyanins. Syrups from various cultivars differed in color stability. Degassing of syrups prior to storage did not enhance color stability. Day-light storage of syrups lowered half-life of Hunter a' values by 10-30% compared with dark storage. Storage in oxygen permeable packaging materials reduced Hunter a' half-life values up to 90%. Hue values should be used rather than absorbance ratios A_{520}/A_{420} to judge color quality. Hunter a' values should be above 50 and hue values below 40-45° to ascertain acceptable color.

INTRODUCTION

ANTHOCYANINS, the pigments responsible for color in most fruits and berries, degrade easily according to various reaction mechanisms as reviewed by Markakis (1982) and Ribéreau-Gayon (1982). The degradation is influenced by oxygen, ascorbic acid, light, pH and temperature, Anthocyanin-containing food products like jams, purée, syrups and juices are therefore susceptible to color deterioration, the visible image changing from a natural red or purple to a more dull brownish color (Abers and Wrolstad, 1979; Spayd and Morris, 1981a; Simard et al., 1982). Anthocyanin degradation has been shown to follow first order reaction kinetics (Meschter 1953; Wrolstad et al., 1970; Debicki-Pospisil et al., 1983).

The color of free anthocyanins is pH dependant, the pigments have their maximum color intensity about pH 1 and are nearly colorless at pH values of about 4.5. A pH-differential method is therefore frequently used for quantification (Fuleki and Francis, 1968). However, the decrease in free anthocyanins actually precedes the development of visual discoloration and determination of anthocyanins by the pH-differential method is not suitable for evaluating visual color (Abers and Wrolstad, 1979; Ribéreau-Gayon, 1982; Skrede et al., 1983). In red wine, Somers (1971) has shown that stable polymeric pigments are formed from anthocyanins during storage. These polymers are responsible for a great proportion of the color intensity in stored wine and are not included in the pH-differential anthocyanin determination.

Hunter color parameters have previously proved valuable in describing visual color deterioration in anthocyanin-containing products (Poei-Langston and Wrolstad, 1981; Spayd and Morris, 1981b; Sistrunk and Gascoigne, 1983; Skrede et al., 1983). The present work was undertaken to study how this method could be used for practical evaluation of color stability in blackcurrant syrups, and to establish limits for acceptable color quality. A further aim was to study the extent that variation in blackcurrant raw material and practical processing and storage conditions influenced color stability.

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MATERIALS & METHODS

BLACKCURRANT SYRUPS from the cultivars Silvergieter, Ben Nevis and Ben Lomond were prepared on a semi-industrial scale during two preceeding years (Blom and Skrede, 1984). The syrups were packed in uncolored glass bottles and stored at 20°C. The first year, half of each syrup was degassed before filling. The samples were stored in a room with south-facing windows or in the dark. The second year, syrups were stored in artificial light (400 lux, Osram warm white) 10 hr per 24 hr.

An industrially prepared blackcurrant syrup, packed in four different packaging materials glass, polyester (PET), polyvinyl chloride (PVC), and high-density polyethylene (HDPE), were stored under controlled conditions as described previously (Skrede et al., 1983).

At various time intervals during the storage experiments total anthocyanin content (pH-differential method), absorbance ratios A_{520}/A_{420} and Hunter L', a', b' and hue values (hue is the angle with tangent b'/a') were determined as reported previously (Skrede et al., 1983). Hunter L', a', b' values were calculated from CIE XYZ tristimulus values obtained from the transmission spectra (Shimadzu 300-UV, Sesakusho Ltd., Kyoto, Japan), using the weighted-ordinate method between 380 and 760 nm (10 nm intervals) (Hunter, 1975). $L' = 10\sqrt{Y}$, $a' = 175 (1.02 X/Y-1)$, $b' = 70 (1-0.847 Z/Y)$. Lightsource C was used for calculation. Before analyses the syrups were diluted 1:5 with water resulting in soluble solid contents of 12° Brix. The pH of the syrups were 3.0-3.2.

Five samples of different commercial syrups were obtained from a processing company and from an official control laboratory. The syrups had been judged by sensory panels at their laboratories to be close to unacceptability. The syrups were used to set acceptability limits for color quality in blackcurrant syrups.

All analyses were made in duplicates. For statistical evaluation, D values at 5% significance level were calculated according to Snedecor and Cochran (1967). D value is the difference required between two means to judge them as significantly different.

RESULTS

Characteristics of syrups in storage experiments

Initial color parameters of blackcurrant syrups from the three cultivars varied between the two growing seasons (Table 1). On average, Silvergieter and Ben Nevis syrups were higher in anthocyanin concentration and Hunter a' values and lower in Hunter L' values than syrups from the Ben Lomond cultivar. Differences in Hunter b' and hue values were not significant.

The industrially prepared blackcurrant syrup was relatively low in anthocyanin concentration (Table 1). Hunter a' and b' values were low while Hunter L' values were correspondingly high. Hue value was comparable to those of the cultivar syrups.

Kinetics of color changes

Semilogarithmic plots of percent residual anthocyanins during long-term storage of the 1981 syrups and the industrial syrup were linear (Fig. 1), showing that decreases in free anthocyanin in blackcurrant syrups followed first order reaction kinetics during storage. At the end of the storage period, anthocyanin concentration approached the detection limit of the analytical method and half-life calculations were based on pigment losses during the initial 18 months of storage (Table 2). The half-life value of Ben Nevis syrup

Table 1—Initial content of free anthocyanins, Hunger L', a', b' and hue values in blackcurrant syrups

	Anthocyanins mg/100g	Hunter			
		L'	a'	b'	Hue°
1981					
Silvergieter	79	25.9	164.1	67.8	22.4
Ben Nevis	79	27.9	171.3	68.2	21.7
Ben Lomond	61	31.6	150.0	66.5	23.9
1980					
Silvergieter	110	29.1	176.5	63.7	19.8
Ben Nevis	82	31.0	177.7	67.4	20.7
Ben Lomond	88	32.8	166.7	66.7	21.9
An industrial syrup	44	48.2	121.0	56.6	23.0
$\overline{D}_{5\%}$	7	2.6	8.2	3.6	—

Table 2—Half-life, $t_{1/2}$, (months) of free anthocyanin contents and Hunter a' values of blackcurrant syrups prepared from different cultivars and from different package types stored at 20°C under artificial light

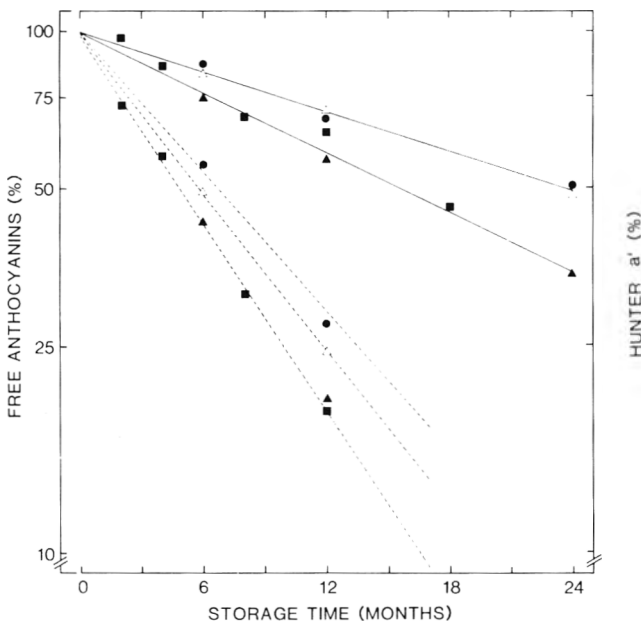|	$t_{1/2}$ (months)	
	Anthocyanins	Hunter a'
Cultivar		
Silvergieter	5.9	23.8
Ben Nevis	6.8	24.0
Ben Lomond	5.0	14.8
An industrial syrup	4.7	16.2
Packaging material		
Glass	4.7	16.2
Polyester	3.4	12.5
Polyvinyl chloride	2.7	8.6
High density polyethylene	0.9	1.9
$\overline{D}_{5\%}$	1.4	3.7

Fig. 1—Semilogarithmic plot of changes in free anthocyanin contents (- - -) and Hunter a'-values (——) in four blackcurrant syrups during storage at 20°C under artificial light: (Δ) Silvergieter; (●) Ben Nevis; (▲) Ben Lomond; (■) an industrial syrup.

Fig. 2—Hue values in four blackcurrant syrups during storage at 20°C under artificial light: (Δ) Silvergieter; (●) Ben Nevis; (▲) Ben Lomond; (■) an industrial syrup.

was higher than that of Ben Lomond and the industrial syrup. This means that the Ben Lomond and the industrial syrup had lower stability of free anthocyanins than the Ben Nevis syrup. Silvergieter seemed to have an intermediate anthocyanin stability among the syrups tested.

Decreases in Hunter a' values also followed first order reaction kinetics throughout the storage period (Fig. 1). Half-life values of the Hunter a' values of Silvergieter and Ben Nevis cultivars were equal and significantly higher than the half-life of the Ben Lomond and the industrial syrup (Table 2). With Hunter L' and b' values, linear relationships in the semilogarithmic plots were found only during the first part of the storage period. Storage beyond 12 months resulted in lower Hunter L' values and higher Hunter b' values than would be expected with first order reaction kinetics. Hunter b' values of Silvergieter and Ben Nevis syrups deteriorated less and Hunter L' values increased more than the corresponding values of Ben Lomond and the industrial syrup.

The different changes in Hunter a' and b' values caused hue values to increase during storage (Fig. 2). Differences in
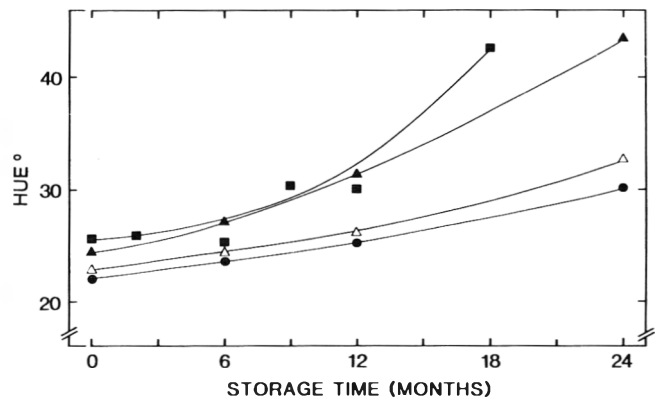
hue values between syrups were more pronounced at the end of the storage period. The Ben Lomond and the industrial syrup gave the highest hue values.

The absorbance ratio A_{520}/A_{420} decreased as the storage proceeded. However, the differentiation between syrups became poorer towards the end of the storage period (Fig. 3). Neither hue values nor absorbance ratios applied to first order reaction kinetics during storage.

Effect of storage conditions

Packaging material greatly influenced the free anthocyanin deterioration in blackcurrant syrups (Fig. 4a). In the industrial syrup packed in four different packaging materials, first order kinetics were generally followed until 10 mg anthocyanins/100g syrup or about 20% of the initial anthocyanin content remained. Correspondingly, linearity in Hunter a' values was found as long as the values exceeded 25% of the initial levels (Fig. 4b). From this point, Hunter a' values decreased more slowly. Half-life values of free anthocyanin content and Hunter a' values of the industrially prepared blackcurrant syrup stored in various packaging materials are shown in Table 2. Both color parameters were best maintained in glass bottles where half-life was about 35% longer than in PET bottles and about 80% longer than in PVC bottles. HDPE clearly was the least suited packaging material for blackcurrant syrups.
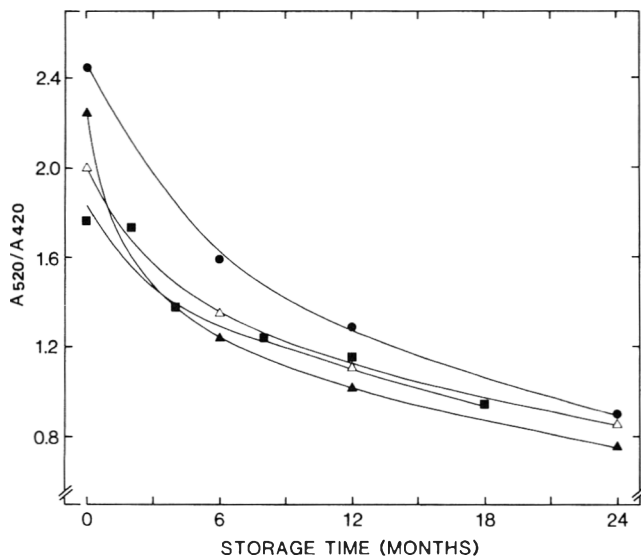
Fig. 3—Absorbance ratio in four blackcurrant syrups during storage at 20°C under artificial light: (△) Silvergieter; (●) Ben Nevis; (▲) Ben Lomond; (■) an industrial syrup.

Hue values were greatly influenced by the packaging material used during storage, again demonstrating the superior storage ability of glass bottles (Fig. 5). Absorbance ratio A_{520}/A_{420} also discriminated between packaging materials of syrups but to a smaller extent than the hue values, and the results are not presented.

The various processing and storage conditions applied in the storage experiments to some extent influenced color parameters. Assuming first order kinetics throughout the storage period of 9 months (Fig. 1), half-life values were calculated (Table 3). Differences in half-lives of free anthocyanins were not significant, while significant differences in half-lives of Hunter a' values were found in Silvergieter and Ben Nevis syrups. Syrups stored under dark conditions were more color stable than syrups stored in daylight. The unevacuated syrups showed a tendency towards longer half-life than evacuated syrups. Significant differences in hue values with storage conditions were not found during the 9 months storage period.

Acceptability of syrups

The Hunter L', a', b' and hue values and the absorbance ratio A_{520}/A_{420} of five commercial blackcurrant syrups, judged by sensory panels at the industrial laboratories to be in the range of unacceptability, are shown in Table 4. Acceptable syrups had Hunter a' values above 50 and hue values below 38°, while samples judged as not acceptable had Hunter a' values below 43 and hue values above 47°. Absorbance ratio was above 0.8 in the acceptable syrups, below 0.7 in the unacceptable. The Hunter L' and b' values did not show clear acceptability limits and values of acceptable and not acceptable syrups to some extent overlapped.
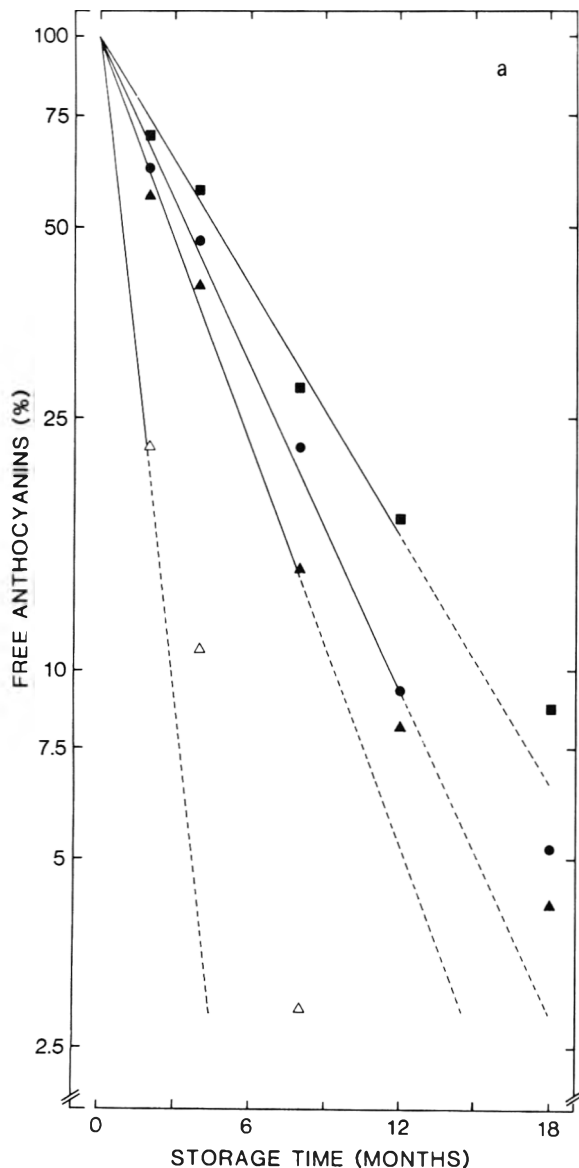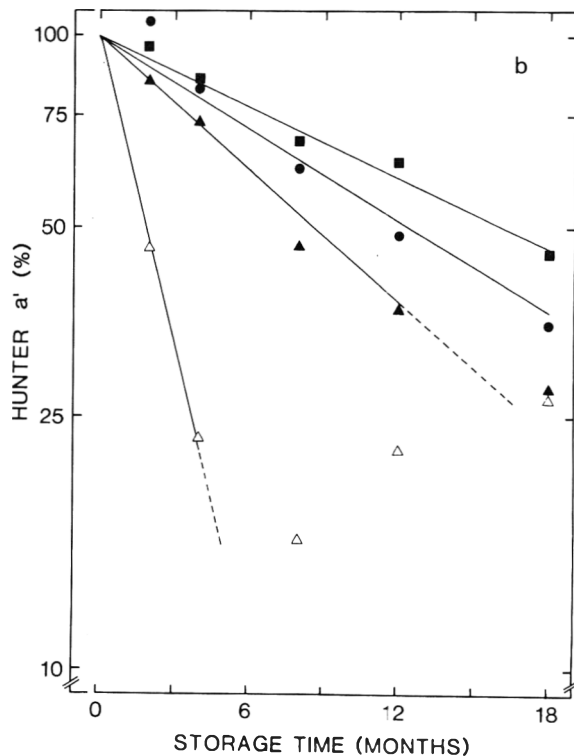
Fig. 4—Semilogarithmic plot of changes in free anthocyanin content (a) and Hunter a'-values (b) in an industrially produced blackcurrant syrup stored at 20°C under artificial light in four different packaging materials: (■) glass; (●) polyester; (▲) polyvinyl chloride; (△) high-density polyethylene.
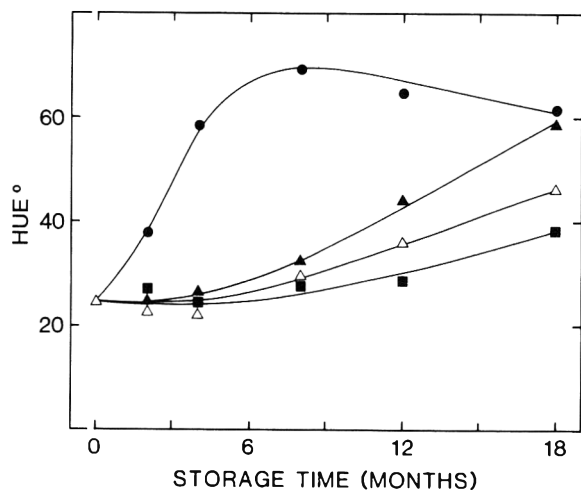
Fig. 5—Hue values in an industrially produced blackcurrant syrup stored at 20°C under artificial light in four different packaging materials: (■) glass; (●) polyester; (▲) polyvinyl chloride; (△) high-density polyethylene.

DISCUSSION

THE CURRENT EXPERIMENTS revealed that first order reaction kinetics can be used when evaluating red color stability in blackcurrant syrups during storage. Despite varying blackcurrant raw materials, first order kinetics applied to the syrups throughout a storage period of two half-life periods. The decrease in Hunter a' values was caused by more extensive changes occuring in the central than in the upper part of the transmission spectrum.

Also disappearance of free anthocyanins followed first order reaction kinetics, in accordance with previous findings (Spayd and Morris, 1980). However, the half-life values calculated for anthocyanins were considerably lower than those of Hunter a' values. As much as 50% of the red color remained in the syrup at the time when free anthocyanins were hardly detectable. Other components, like polymeric compounds formed from anthocyanin monomers, must therefore contribute to red color during the later periods of storage (Somers, 1971). Since the decrease in red color partly levels off as the amount of free anthocyanins disappears, these polymeric pigment forms must have a higher stability compared with the monomeric form.

The changes in Hunber b' values with storage were initially caused by increased transmission in the lower and central part of the syrup spectrum, resulting in decreased Hunter b' values. Upon further storage, transmission in the lower part of the spectrum (400-520 nm) decreased, with that in the central part (520-570 nm) remaining fairly constant. This resulted in increased Hunter b' values during the later part of the storage period. Thus, Hunter b' values cannot be used directly to predict color stability in blackcurrant syrups during storage to the same extent as Hunter a' values. However, the parameter is important since the overall color impression of the syrup depends on the relative amount of red and yellow. In all syrups the ratio of yellow to red, expressed by the hue value, increased during storage. This corresponds to a more yellow hue of the blackcurrant color upon storage. Similarly, Abers and Wrolstad (1979) and Spayd and Morris (1981a) found strawberry preserves to attain an color more yellow in hue with storage.

By proper definition of color acceptability levels, the present quality of any actual syrup may easily be evaluated from its hue value. In the present investigation an upper acceptability limit of 40-45° was found for the hue value. This is somewhat higher than the limit of 35° set previously

Table 3—Half-life values, t½, (months) of free anthocyanins and Hunter a' values of blackcurrant syrups during storage in daylight and in the dark. Hue values after 9 months storage

	t½ (months)		
	Anthocyanins	Hunter a'	Hue°
Silvergieter			
Unevacuated			
daylight storage	5.0	31.9	21.3
dark storage	5.8	41.8	20.7
Evacuated			
daylight storage	4.4	31.8	20.4
dark storage	5.3	45.2	20.6
Ben Nevis			
Unevacuated			
daylight storage	4.3	23.9	19.5
dark storage	5.0	27.8	19.8
Evacuated			
daylight storage	3.3	17.0	20.6
dark storage	4.1	25.5	19.7
Ben Lomond			
Unevacuated			
daylight storage	4.2	24.8	20.5
dark storage	4.7	27.3	20.6
Evacuated			
daylight storage	3.9	21.8	20.2
dark storage	4.4	24.5	20.8
$\overline{D}_{5\%}$	1.2	7.4	—

Table 4—Hunter L', a', b' values and hue values of five industrially prepared blackcurrant syrups. The syrups were judged by the suppliers to have sensory color quality just over or below the acceptability limit

	Hunter			
Evaluation of sample	L'	a'	b'	Hue°
Acceptable	56.1	50.5	39.4	38.0
Acceptable	48.2	85.5	50.6	30.6
Acceptable	51.7	69.4	50.5	36.0
Not acceptable	55.5	43.1	47.2	47.6
Not acceptable	62.7	36.3	41.5	48.8

(Skrede et al., 1983). The settings indicate the present Swedish and Norwegian attitude towards color acceptability in blackcurrant syrups.

The extensively used absorbance ratio A_{520}/A_{420} (Morris and Spayd, 1980; Sistrunk and Gascoigne, 1983) proved to be less suited for color evaluation than the hue value, since the differentiation between samples became less distinct at colors close to the acceptability limit. This may be the explanation to the hue values appearing to predict visual color deterioration in the syrups slightly better than the absorbance ratio (Skrede et al., 1983). It is reasonable that hue values, containing information from the entire visible region, give better color information than an absorbance ratio based on only two distinct wavelengths.

The most extensive effect on both free anthocyanin and color stability during storage of blackcurrant syrup was caused by the packaging materials and hence by the oxygen availability (Skrede et al. 1983). The detrimental effect of oxygen to anthocyanin stability has been well documented previously (Nebesky et al., 1949). Degassing of syrups prior

Purification and characterization of a
Clostridium perfringens α-galactosidase

TIMOTHY DURANCE and BRENT SKURA

──────────── ABSTRACT ────────────

Of 21 strains of *C. perfringens* tested for hydrolysis of α-galacto-
sides, 10 utilized either raffinose or melibiose while 2 utilized both
sugars. Spore production in Duncan Strong medium was superior in
the presence of raffinose as opposed to starch in 12 out of 21
strains. *C. perfringens* M34 yielded 1.2 units of α-galactosidase/g
washed cells. This enzyme had an isoelectric point of 5.6 and a pH
optimum for hydrolysis of PNPG of 6.3. Native and monomer
molecular weights were 96,000 and 46,000 daltons respectively.
Km was 0.20 ± 0.02mM PNPG. Heat stability of the enzyme de-
creased as purity increased. This trend was partially reversed by
addition of 2-mercaptoethanol, NADH, cysteine, and/or bovine
serum albumin to reaction mixture.

INTRODUCTION

LEGUMES are widely consumed as low cost sources of
dietary protein. Excessive flatulence, however, long asso-
ciated with legume consumption, may limit the accepta-
bility of legumes in the diet. Several studies have shown
that the α-galactoside sugars raffinose and stachyose con-
tribute to flatulence (Murphy et al., 1972; Rackis et al.,
1970; Steggerda et al., 1966). These sugars are not hydro-
lyzed or absorbed by the mammalian digestive system
(Taeufel et al., 1965). The sugars therefore pass into the
lower bowel, where they may be fermented by various
resident bacteria, with the production of gas. Strains of
Clostridium perfringens in particular have been implicated
as major sources of intestinal gas (Richards et al., 1968;
Sacks and Olson, 1979).

Some studies have suggested that other factors besides
α-galactosides may contribute to flatulence (Calloway,
1973; Calloway et al., 1971; Wagner et al., 1976). An α-
galactosidase similar or identical to the bacterial enzyme
present in the large intestine could be a valuable research
tool for clarifying the role of these sugars in flatulence.
The objective of this study was to isolate and characterize
the α-galactosidase of *C. perfringens* (α-GALCp).

MATERIALS & METHODS

Microorganisms

Twenty strains of *C. perfringens*, isolated from fecal and non-
fecal sources were donated by the Division of Laboratories, British
Columbia Ministry of Health. One other strain was isolated from a
soil sample. All were identified by the method of Hauschild, 1975.
Strain M34 was confirmed to be *C. perfringens* by means of the API
Anaerobe identification system (Analytab Products, Plainview, NY).

Strains were maintained as spore suspensions in distilled water
at 4°C. Spores were induced and counted by the methods of Labbe
and Rey (1979), employing either raffinose or starch as the sporula-
tion carbohydrate. Short term culture maintenance utilized Cooked
Meat medium (Difco).

Strains capable of producing acid and gas within 36 hr at 45°C
from 2.0% trypticase peptone (BBL), 0.35% agar, 0.002% phenol

red and 0.5% carbohydrate were considered positive for that carbo-
hydrate.

For production of α-GALCp, cultures were grown at 42°C in
α-Galactoside Broth (1.5% trypticase peptone, 0.5% proteose pep-
tone, 0.5% sodium chloride, 0.2% dipotassium phosphate, and 0.1%
sodium thioglycollate, with or without 0.5% raffinose and 0.5%
melibiose). The ferementation procedure was as follows. Spore sus-
pension was heat shocked (75°C; 20 min) and surface plated onto
Germination Agar (1.5% trypticase peptone, 1.0% yeast extract,
1.5% agar). Germination plates were incubated overnight at 42°C.
Single colonies were inoculated into Cooked Meat medium, incu-
bated 24 h, and a loopful of culture used to inoculate 15 mL α-
Galactoside Broth (with or without α-galactosides). This culture was
incubated overnight, then used as the inoculum for 125 mL α-
Galactoside Broth, which in turn, after 6 h incubation, was the
inoculum for 2,500 mL α-Galactoside Broth. Following germination
of the spores, no special steps were taken to ensure anaerobiosis,
except that freshly autoclaved media was used throughout and cul-
tures were grown without shaking or mixing.

Purification of α-GALCp. Cells harvested by centrifugation
(9,000 x g; 4°C), washed with 3 volumes of buffer, (0.05M mono-
potassium phosphate, pH 6.7) and resuspended in the same buffer,
were disrupted by passage thorough a cold Aminco-French Pressure
Cell (Silver Spring, MD), at 15,000 psi and 2°C. The crude extract
was clarified by centrifugation (27,000 x g; 30 min; 4°C).

DEAE cellulose chromatography

Crude extract (100-500 units α-GALCp) was applied to a 2.5 cm
x 22 cm column of Whatman DE-32 DEAE cellulose (Whatman
Chemical Seperation Ltd., England), previously equilibrated with
0.02M monopotassium phosphate buffer, pH 6.7. The column was
washed with 300 mL starting buffer and the sample was eluted with
a 0.02–0.5M sodium chloride gradient (total volume. 840 mL).
Active fractions were pooled and concentrated by ultrafiltration at
4°C in an Amincon (Lexington, Mass.) Model 52 cell fitted with a
Diaflo PM-10 membrane.

Gel filtration chromatography

Gel filtration was carried out at 2°C in a 2.6 cm x 92 cm column
of Sephacryl S400 (Pharmacia, Inc., Dorval, Que.), equilibrated with
0.05M potassium phosphate buffer, pH 6.7. Fractions (6 mL) were
collected and assayed for α-galactosidase activity and absorbance at
280 nm. Molecular weight estimations were made by comparison
with protein standards catalase (liver), bovine serum albumin (BSA),
and ovalbumin, all supplied by Sigma (St. Louis, MO). Calculations
were as described by Freifelder, (1976).

Enzyme activity

α-Galactosidase activity was monitored by means of a fixed time
(15 min) assay at 45°C. The reaction mixture contained 0.67mM
p-nitrophenyl alpha-D-galactopyranoside (PNPG), 0.06M sodium
phosphate buffer (pH 6.5), 1.5 mg BSA/mL, 3mM cysteine, and an
appropriate amount of enzyme. The reaction was stopped by the
addition of 5 volumes 0.1M sodium carbonate and the absorbance
read at 405 nm. One unit of enzyme was defined as the amount
required to hydrolyse 15 umole PNPG in 15 min at the specified
temperature. The absorbance of 1 μg p-nitrophenol/mL was 0.124.

Electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis
(SDS-PAGE) was carried out in a 12 cm x 12 cm vertical slab
electrophoresis unit (Atta Inc., Japan) by the method of Laemmli
(1970). Protein bands were located, by means of a Coomassie blue
stain consisting of 27% isopropanol, 10% acetic acid, and 0.04%

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Coomassie brilliant blue R-250 (BioRad, Richmond, CA) in water. Gels were stained overnight, then destained with 12% isopropanol and 7% acetic acid in water. Calculation of corresponding molecular weights was by the method described by Weber and Osborn (1969).

Isoelectric focusing

Analytical horizontal polyacrylamide gel isoelectric focusing (IEF-PAGE) was carried out in a Bio-Rad Model 1415 electrophoresis cell, according to the manufacturers instructions. Gel slabs were 45 mm × 125 mm and either 0.8 or 1.6 mm thick. Bands were located by means of a Coomassie blue protein stain and/or by a PNPG α-galactosidase activity stain in which the gel was placed on filter paper and flooded with the solutions described in the enzyme assay. Following incubation the reaction was stopped by flooding the gel with sodium carbonate solution. The pH gradient was measured with a surface pH electrode (0.5 cm probe; Corning Sci. Products, Medfield, MA) and a Fisher Accumet pH meter, Model 620.

Thermal stability

Duplicate 0.1 mL samples of purified enzyme solution were incubated for 15 min at various temperatures in 12 mm × 10 mm test tubes. Test tubes were pre-equilibrated in water baths at the test temperatures and experiments were staggered to ensure that incubation times were equivalent to within 2 sec. The enzyme was then immediately assayed against PNPG at 35°C. Relative activity was determined by comparing activity to that of enzyme incubated at 2°C for a corresponding period of time and assayed against PNPG at 35°C.

Substrate affinity

Fixed time assays (1 min) were used to estimate initial velocities of enzyme substrate reactions (pH 6.5; 40°C; enzyme concentration 8.0 μg protein/mL) at 6 PNPG concentrations ranging from 0.12 to 3.0 mM. Km, Vmax, and standard errors for each were computed using the non-linear regression program of Oestreicher and Pinto, 1983.

Protein determinations

Protein determinations were done by the method of Lowry et al. (1951) as modified by Peterson (1977). Crystalline BSA (Sigma) was used as a standard.

RESULTS & DISCUSSION

Strain selection

The ability of C. perfringens to produce acid and gas from fructose, sucrose, raffinose, and melibiose is illustrated in Table 1. Of the 21 strains examined, 2 strains demonstrated rapid utilization of both raffinose and melibiose. One of these, designated M34, was used for further study of the α-GALCp.

Sporulation

Sporulation of C. perfringens is frequently difficult to induce and many complex media have been developed for this purpose. Of these the medium of Duncan and Strong (1968) has gained the widest acceptance. Labbe and Rey (1979) reported that replacement of the starch in Duncan Strong medium with raffinose improved spore recoveries in 6 of 8 C. perfringens strains tested. In this study 12 of 21 strains gave higher spore counts with raffinose (Table 2). Neither carbohydrate, however, was preferred by all strains and one strain failed to sporulate in either medium.

Yields of α-galactosidase

When C. perfringens M34 was grown in broth containing 0.5% raffinose and 0.5% melibiose, the yield of α-GALCp in the crude cell extract was 1.2 U/g washed cells (wet weight). Cell free growth medium contained little or no α-GALCp activity. When the strain was grown without exposure to α-galactosides, α-GALCp in the crude cell extract

Table 1—Production of acid and gas from carbohydrates by strains of C. perfringens

Strain	Substrate			
	Fructose	Sucrose	Raffinose	Melibiose
F1	+	+	+	−
F2	+	−	−	−
F4	+	+	−	−
F5	+	+	−	−
F7	+	+	−	−
F0	+	+	+	+
FA	+	+	+	−
M06	+	+	−	−
M20	+	+	+	−
M21	+	+	+	−
M22	+	+	+	−
M30	+	+	−	−
M31	+	+	−	−
M34	+	+	+	+
M40	+	+	−	−
M64	+	+	−	+
M74	+	+	−	−
M75	+	+	+	−
M81	+	+	+	−
M92	+	+	−	+
M24R	+	+	+	−

+ = Acid and gas produced; − = no acid or gas produced.

Table 2—Spore counts of C. perfringens strains grown in Duncan Strong sporulation medium (DS) with starch or raffinose as the carbon source (thousands of spores/mL)

Strain	DS with starch	DS with raffinose
F0	320	12.1
F1	400	N.D.[a]
F2	140	1450
F4	40	6.7
F5	52	1580
F7	510	230
FA	6200	2400
M06	169	170
M20	10	160
M21	N.D.[a]	24
M22	330	1900
M30	0.02	365
M31	2.7	5.4
M34	800	98
M40	0.18	244
M64	N.D.[a]	16
M74	N.D.[a]	N.D.[a]
M75	250	0.02
M81	2600	2.2
M92	N.D.[a]	1800
M24R	0.80	82

[a] N.D. = none detected.

was only 0.07 U/mL. Apparently, in this strain at least, α-GALCp is constitutive but partially inducable.

C. perfringens is known for its ability to grow rapidly at 45°C and results presented here indicate that strain M34 was capable of utilizing α-galactosides at that temperature. A temperature of 42°C was chosen for production of the enzyme because of the possibility that genetic control of α-GALCp might be plasmid mediated. Whether this is the case in C. perfringens has not been determined, but it has been shown to be the case for an α-galactosidase of Escherichia coli K12 (Schmid and Schmitt, 1976). C. perfringens has been shown to harbor a variety of plasmids (Duncan et al., 1978). Growth at 46°C has been suggested as a way of "curing" C. perfringens of plasmids (Rood et al., 1978). In light of the possibility of plasmid control of

the enzyme, it was thought prudent to use a lower temperature to grow the organism.

Purification of α-GALCp

The results of the purification procedure are summarized in Table 3. Cell free crude extract, applied to a DEAE cellulose column and eluted with a linear sodium chloride gradient gave a single peak of α-GALCp activity at a salt concentration of 0.19M (Fig. 1). The active fractions were pooled, concentrated by ultrafiltration, and applied to a Sephacryl S400 column. Active fractions were again pooled, concentrated, and reapplied to the same column. The pooled activity peak from this column represents the highest degree of purity acheived. SDS-PAGE of this fraction indicated the presence of one major band and at least two weaker protein bands (Fig. 2).

Further attempts to improve purity were abandoned because of poor recovery of activity. Attempts to chromatograph α-GALCp on Sephadex G150 at room temperature resulted in loss of ⩾ 60% of the activity. A final attempt to improve purity by reapplying the sample to DEAE cellulose resulted in a loss of greater than 90% of the enzyme activity. Therefore further characterization was carried out with the Sephacryl S400(2) active fractions.

pH optimum

Relative rate of hydrolysis of PNPG was greatest at pH 6.3 (Fig. 3). The pH optimum of α-GALCp in the crude extract was similar.

Molecular weights

The native molecular weight of α-GALCp, as estimated by means of gel filtration on Sephacryl S400, was approximately 96,000 daltons (Fig. 4). Although it was not possible to positively identify the enzyme band on SDS-PAGE,

the most prominent band corresponded to a molecular weight of 46,000 daltons. On this basis, the native protein appears to be a dimer.

Isoelectric point

The enzyme was focused on a pH 4.0 to pH 6.5 gel gradient and located by means of an activity stain, at pH 5.6 ± 0.1. No other band of activity was noted.

Affinity for PNPG

Km and Vmax, plus or minus their standard errors were calculated to be: Km = 0.20 ± 0.02 mM; Vmax = 2.02 ± 0.06 μM/min. A Lineweaver Burk plot of the same data is included to allow a visual estimate of goodness of fit (Fig. 5).

Activation of α-GALCp by various compounds

Loss of enzyme activity was a recurrent problem throughout the isolation procedure. Gel filtration at room temperature typically resulted in loss of at least 60%. Chromatography at 2°C improved recoveries. The microbial inhibitor sodium azide (0.02%) and to a lesser extent chlorhexidine gluconate (0.002%) also decreased activity (Table 4). In assays at 45°C, 2-mercaptoethanol, NADH, cysteine, and BSA each increased the apparent activity of α-GALCp. The combination of BSA and cysteine had the

Table 3—Effects of purification steps on specific activity and yield of α-GALCp

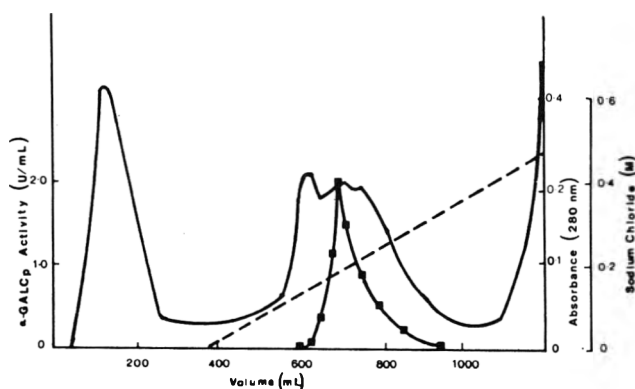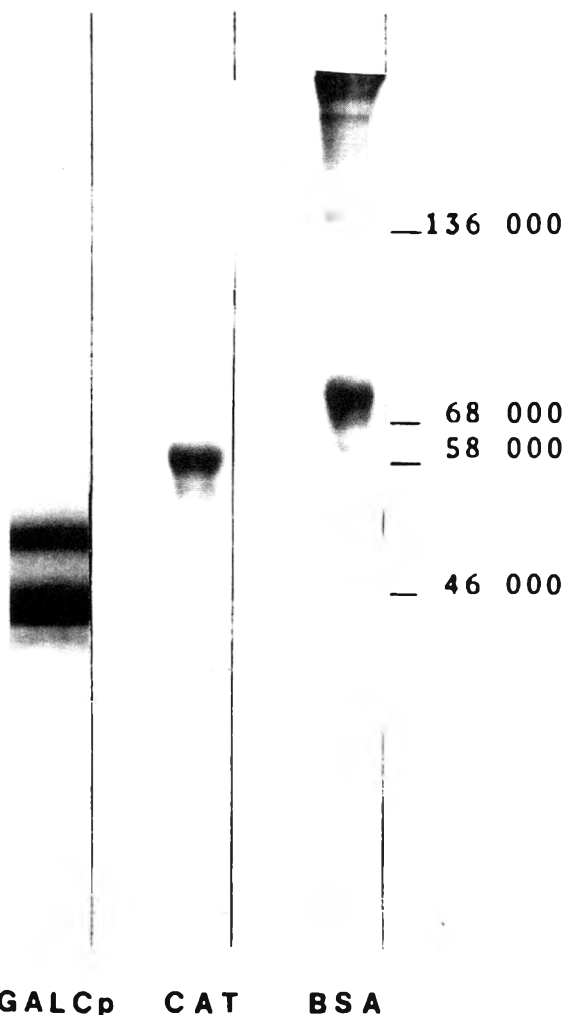| Procedure	Activity (U/mL)	Specific activity (U/mg protein)	Yield (%)
French Press.	1.21	0.17	100
DEAE Cellulose	0.72	0.81	64
Sephacryl S400	0.18		50
Sephacryl S400 (2)	0.13	1.61	32

Fig. 1—DEAE cellulose chromatography of α-GALCp: Absorbance at 280 nm, (—); α-galatosidase activity, (■); sodium chloride concentration, (- - -).

α-GALCp   CAT   BSA

Fig. 2—SDS-PAGE of α-GALCp, catalase, and bovine serum albumin (BSA).
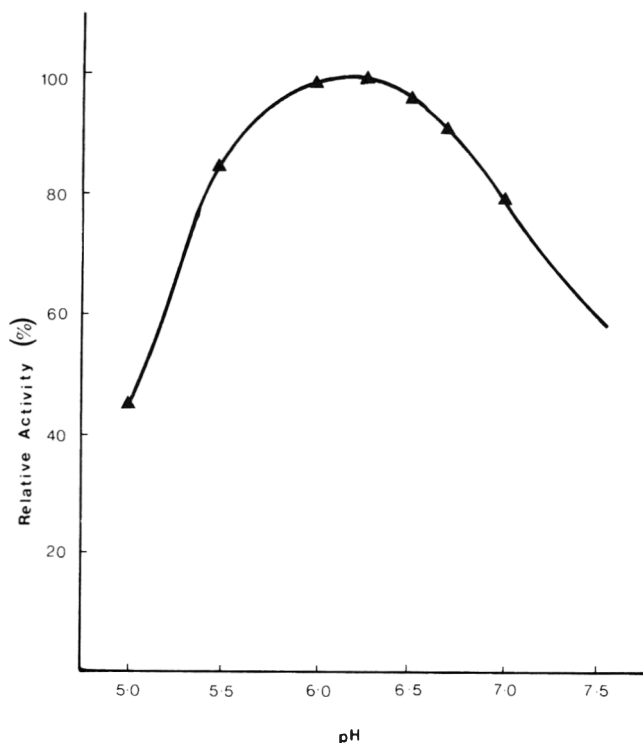
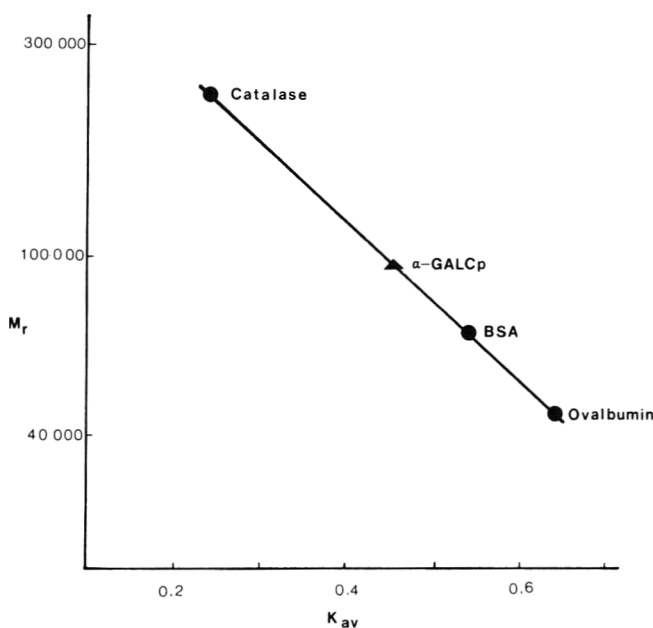Fig. 3—Optimum pH of α-GALCp activity for hydrolysis of PNPG at 30°C.

Fig. 4—Gel filtration of α-GALCp and protein standards on Sephacryl S400.

Fig. 5—Lineweaver-Burk plot of α-GALCp hydrolysis of PNPG at pH 6.5, 40°C, and enzyme concentration of 8 μg/mL; (min μM^{-1} vs mM^{-1}).

Table 4—Effects of various compounds on α-GALCp activity at 30°C and 45°C. 100% Relative activity was activity at a given temperature in assay buffer without additives

Compound	Conc. (mM)	Relative Activity (%) 30°C	Activity (%) 45°C
Sodium azide	3.0		76
Chlorhexidine gluconate	0.03		89
BSA	0.02	103	144
NADH	1.3	97	120
Cysteine	3.0	102	114
Mercaptoethanol	100	96	122
BSA-NADH	0.02, 1.3		156
BSA-cysteine	0.02, 3.0		163
BSA-mercaptoethanol	0.02, 100		151

greatest effect and was used in routine assays. In an attempt to elucidate the mechanism of activity enhancement by these compounds, their effect on activity at 30°C was examined. At this lower temperature no activation was noted.

Other authors have noted the activation effect of 2-mercaptoethanol and NADH on the α-galactosidase of *E. coli* K12 (Burstein and Kepes, 1971). The effect of BSA was examined because it had been described as improving the heat stability of the α-galactosidase of *Bacillus stearothermophilus* (Pederson and Goodman, 1980). It w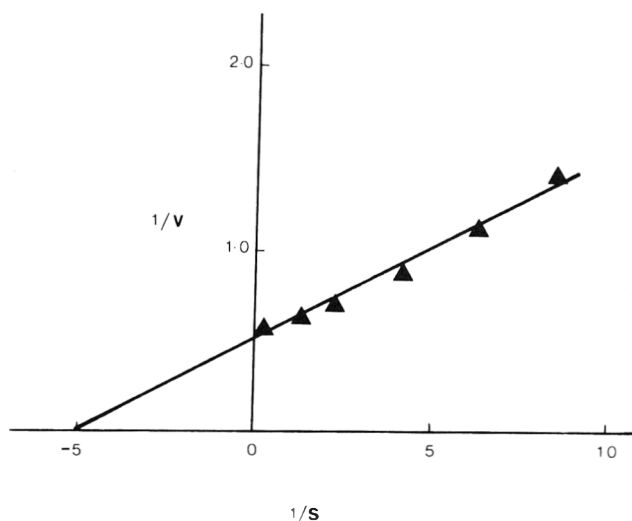ould appear that in the case of α-GALCp the reducing environment and/or the presence of the protein in the form of BSA, serve to increase the apparent activity of the enzyme.

Nonetheless, the question remains as to whether the reducing environment alters the active site of the enzyme, or whether it serves to stabilize the enzyme against thermal denaturation. If the former was the case one would expect activity to be increased at all temperatures, while in the latter case one would expect to see an effect only at temperatures sufficiently high to initiate thermal denaturation. Since the reducing compounds did not increase apparent activity at 30°C but did at 45°C, it is likely that the mechanism of activation is improvement of thermal stability.

Heat stability

Heat stability of α-GALCp decreased markedly as purity increased. The enzyme in the crude extract had an apparent temperature optimum of 47°C in a 15 min assay while the purified enzyme had an optimum of 43°C (Fig. 6). The purified enzyme however, was 96% inactivated by 15 min at 45°C (Fig. 7). This fact very likely accounts for at least some of the apparent enzyme losses during the purification procedure, as the routine assay temperature during purification was 45°C. The unexpectedly poor heat stability demonstrated for the purified enzyme (Fig. 7) made interpretation of the results of the enzyme assays more difficult, since some heat denaturation may have occurred during the assay period in spite of the addition of activators BSA and cysteine to the assay mixture. In an attempt to address this
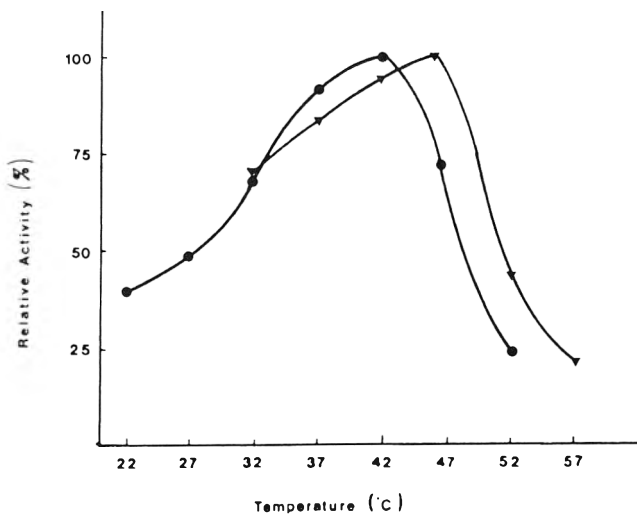
Fig. 6—*Temperature optimums of α-GALCp in crude extract (▼) and in α-galactosidase active fractions of Sephacryl S400 (2) chromatography (●). Each point represents the average of duplicate determinations.*

Fig. 7—*Thermal stability of α-GALCp, incubated at various temperatures for 15 min, then assayed at 35°C.*

problem enzyme activity was reported in terms of the entire assay period. Thus one unit of activity defined here as the enzyme required to hydrolyze 15 μmoles PNPG in 15 min is not equivalent to the conventional unit of activity which is expressed as hydrolysis of 1 μmole PNPG/min.

CONCLUSIONS

THIS STUDY has reported the partial purification and characterization of α-galactosidase from *C. perfringens*. The enzyme has an apparent molecular weight of 96,000 daltons, an isoelectric point of pH 5.6, and a pH optimum of 6.3. Km was 0.20 mM PNPG. Information on this enzyme may be useful in elucidating the mechanism of gas production *in vivo*. Also the enzyme itself could prove to be a valuable research tool for *in vitro* and animal studies of the flatulence potential of legume components. The *C. perfringens* α-galactosidase, however, would be unsuitable for the treatment of foods for human consumption because of the pathogenic nature of *C. perfringens*.

REFERENCES

Burstein, C. and Kepes, A. 1971. The α-galactosidase of Escherichia coli K12. Biochim. Biophys. Acta. 230: 52.
Calloway, D.H. 1973. Gas forming properties of food legumes. Proc. of the symposium "Nutritional Improvement of Food Legumes by Breeding." FAO: Rome, Italy, 3-5. July 1972.
Calloway, D.H., Hickey, C.A., and Murphy, E.L. 1971. Reduction of intestinal gas-forming properties of legumes by traditional an experimental food processing methods. J. Food Sci. 36: 251.
Duncan, C.L., Rokos, E.A., Christenson, C.M., and Rood, J.I. 1978. Multiple plasmids in different toxigenic types of Clostridium perfringens: possible control of β-Toxin Production. In "Microbiology — 1978," (Ed.) D. Schlessinger, p. 240. Am. Soc. Microbiology, Washington, DC.
Duncan, C.L., and Strong, E.B. 1968. Improved medium for sporulation of Clostridium perfringens. Appl. Microbiol. 16: 82.
Freifelder, D. 1976. "Physical Biochemistry: Applications to Biochemistry and Molecular Biology," p. 189. W.H. Freeman & Co: San Francisco.
Hauschild, A.H.W. 1975. Criteria and procedures for implicating Clostridium perfringens in food-born outbreaks. Can. J. Public Health. 66: 388.
Labbe, R.G., and Rey, D.K. 1979. Raffinose increases sporulation and enterotoxin production by Clostridium perfringens Type A. Appl. Environ. Microbiol. 37: 1196.
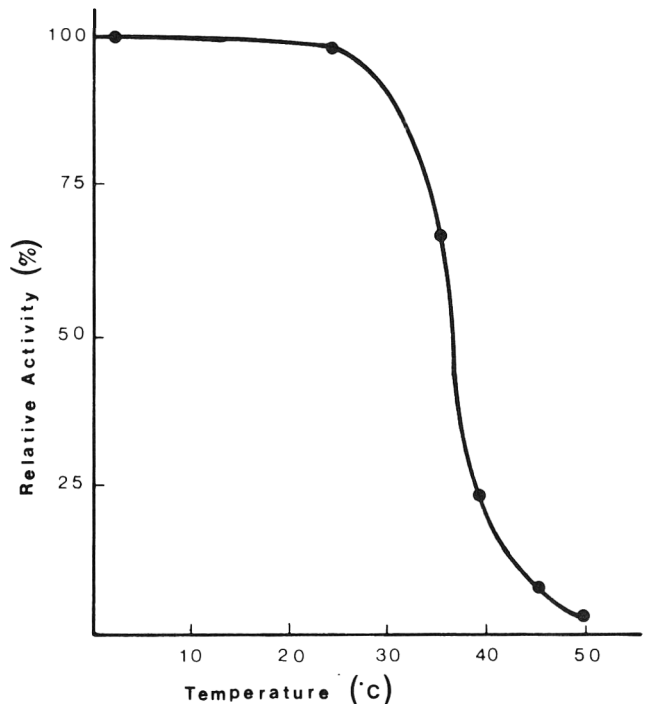Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London). 227: 680.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the Folin reagent. J. Biol. Chem. 193: 265.
Murphy, E.L., Horsley, H., and Burr, H.K. 1972. Fractionation of dry bean extracts which decrease carbon dioxide egestion in human flatus. J. Agric. Food Chem. 20: 813.
Oestreicher, E.G., and Pinto, G.F. 1983. Pocket computer program for fitting the Michaelis-Menton equation. Comput. Biol. Med. 13: 309.
Pederson, D.M. and Goodman, R.E. 1980. Isozymes of αgalactosidase from Bacillus stearothermophilus. Can. J. Microbiol. 26: 978.
Peterson, G.L. 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal. Biochem. 83: 346.
Rackis, J.J., Honig, D.H., Sessa, D.J., and Steggerda, F.R. 1970. Flavor and flatulence factors in soybean protein products. J. Agri. Food Chem. 18: 977.
Richards, E.A., Steggerda, F.R., and Murata, A. 1968. Relationship of bean substrates and certain intestinal bacteria to gas production in the dog. Gastroenterology 55: 502.
Rood, J.I., Scott, V.N., and Duncan, C.L. 1978. Identification of a transferable tetracycline resistance plasmid (pCW3) from Clostridium perfringens. Plasmid 1: 563.
Sacks, L.E., and Olson, A.C. 1979. Growth of Clostridium perfringens strains on α-galactosides. J. Food Sci. 44: 1756.
Schmid, K. and Schmitt, R. 1976. Raffinose metabolism in Escherichia coli K12. Eur. J. Biochem. 67: 95.
Steggerda, F.R., Richards, E.A., and Rackis, J.J. 1966. Effects of various soybean products on flatulence in the adult man. Proc. Soc. Exptl. Biol. Med. 121: 1235.
Taeufel, K. Ruttloff, H., Krause, W., Taeufel, A., and Vetter, K. 1965. Zum Intestinalen Verhalten von Galakto-Oligosacchariden beim Menschen. Klin. Wochenshr. 43: 268.
Wagner, J.R., Becker, R., Gumbmann, R., and Olson, A.C. 1976. Hydrogen production in the rat following the ingestion of raffinose, stachyose and oligiosaccharide-free bean residue. J. Nutr. 106: 466.
Weber, K., and Osborn, M. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244: 4406.
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Response Transformation: An Example

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────────────── ABSTRACT ──────────────

A set of data involving 108 observations from a previous study on a sterilized, concentrated baby formula is reconsidered. Originally, four separate second degree models involving 84 different coefficients were fitted. However, if the response values are transformed from y to ln y, a model containing just five parameters explains 87% of the variation in all of the data about their grand mean. Moreover, this simple equation does not include one of the five predictor variables, a fact not previously apparent. These data provide an example of the effectiveness of a transformation.

INTRODUCTION

IN A PREVIOUS PAPER, Swanson et al. (1967) reported the results of part of an experiment on a sterilized, concentrated, baby formula. Their purpose was to select conditions which would provide a formula with acceptable storage stability for over a year at room temperature, and which would withstand terminal sterilization once the concentrate had been diluted with water. In the present paper, we re-analyze the data using a data transformation. Our objective is to show how astonishingly effective such a change of metric can be, making it possible to summarize the results much more simply than is at first apparent.

MATERIALS & METHODS

SWANSON et al. (1967) examined five predictor variables: (1) Preheating of the milk solids for 25 min at a temperature, F; the range of F was 175 - 205°F (79.4 - 96.1°C). (2) Addition of sodium polyphosphate, P, 0 - 0.14%. (3) Addition of sodium alginate, A, 0 - 0.3%. (4) Addition of lecithin, L, 0 - 1.5%. (5) Addition of carrageenan, C, 0.444 - 0.032%. Three experimental levels were chosen for predictor variables 1 - 4, and five levels for variable 5. Because these levels were selected as equally spaced, they may be coded to (−1, 0, 1) for variables 1 - 4 and (−2, −1, 0, 1, 2) for variable 5. Such coding not only greatly simplifies the handling of the data but, before the experiment is planned, aids the consideration and choice of which experimental points to run. Table 1 shows the coding pattern. Clearly, 3 × 3 × 3 × 3 × 5 = 405 treatment combinations are now defined and could be examined. However, application of standard techniques of experimental design (Box et al., 1978) enables quadratic surfaces to be fitted using only a small fraction of that number. In the event, only the 27 combinations shown in Table 2 were needed. (In the statistical literature, this special type of response surface design is known as a *composite design* and consists of a 2_V^{5-1} fractional factorial design (I = −12345) plus five pairs of axial points at the extreme levels, plus one center point, i.e., a cube plus star (unbalanced in variable 5) plus center point.)

The order in which the experimental runs were carried out by Swanson et al. (1967) is indicated by the numbers in the first column of Table 2. Four response values were obtained at each treatment combination. The response y_t (for t = 0, 3, 6, 9) is the Brookfield viscosity in centipoise after t months. It is determined immediately after opening and without any agitation. To be acceptable, the product viscosity had to be less than 1000 centipoise. Only runs 3, 20, and 24 produced response values exceeding this level, indicating that these runs are situated in an undesirable region of the x-space.

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To each of the four columns of y-values in Table 2, Swanson et al. (1967) fitted a second order quadratic equation. Each column in Table 3 shows the coefficients (rounded to integers to facilitate comparisons) in the appropriate model, using the standard notation

$$\hat{y} = b_0 + b_1 x_1 + \ldots + b_5 x_5 + b_{11} x_1^2 + \ldots + b_{55} x_5^2 + b_{12} x_1 x_2 + \ldots$$

$$+ b_{45} x_4 x_5,$$

where $\hat{y}$ denotes the fitted value of the response, and all terms in the x's of zero, first, or second (quadratic) order are included in the model. (Note: The numbers obtained by Swanson et al. (1967, Table 7) differ from those in our Table 3 simply because those authors used the nonstandard coding 1, 2, 3 for factors 1 - 4 and 0, 1, 2, 3, 4 for factor 5. However if we were to substitute $x_1 = F-2$, $x_2 = P-2$, $x_3 = A-2$, $x_4 = L-2$, and $x_5 = C-3$, in our equations, Swanson et al.'s fitted equations would emerge immediately. So this difference is a technically unimportant one.)

In Table 3, the pattern of statistically significant coefficients varies from response to response in a confusing manner. Using the four response functions directly is a possible, but somewhat cumbersome, way to proceed. Can the analysis be simplified in any way?

One feature that is seen in Table 2 is that the response data cover several orders of magnitude, suggesting that it would be worth checking for a possibly useful transformation, by the Box and Cox (1964) technique, for example. For additional illustrations, see Draper and Smith (1981). The large range of y also raises the possibility that the usual regression assumption of homogeneity of variances may not be valid. Because of the lack of replication, this cannot be checked directly, however.

RESULTS

WHAT TRANSFORMATION is sensible? We considered the family of power transformations $Y = (y^\lambda - 1)/\lambda$. The changes of origin (−1) and of scale (1/λ) are present simply to make the family continuous in λ through λ = 0, where Y = ln y, and they do not affect the basic power nature of the transformation. The parameter λ is chosen by seeking a maximum value for the likelihood function. For all four sets of data, the maximum likelihood values of λ are close to zero, making the ln y transformation a sensible choice for all four responses. (We note in passing that ln y also happens to be the appropriate variance stabilizing transformation when the standard deviation of y is proportional to y.)

Examination of the values of the t-statistics for individual coefficients in the four second order fits to ln y shows that:

Table 1 — Actual levels chosen for experimental variables, and their levels after coding to $x_1, x_2, \ldots, x_5$ [a]

Variable, and designation	Coded level, x_i =				
	−2	−1	0	1	2
Preheating (°F/25 min), x_1		175	190	205	
Sodium polyphosphate, x_2		0	0.07	0.14	
Sodium alginate, x_3		0	0.15	0.30	
Lecithin, x_4		0	0.75	1.50	
Carrageenan, x_5	0.444	0.341	0.238	0.135	0.032

[a] For example, x_1 = (Preheating temperature −190)/15 converts the three temperatures 175°, 190°, 205° F (79.4°, 87.8°, 96.1° C) to −1, 0, 1, respectively.

Table 2—Data from a sterilized, concentrated, baby formula experiment

Run reference no.	Levels of coded predictors					Response values (subscript = storage period in months)			
	x_1	x_2	x_3	x_4	x_5	y_0	y_3	y_6	y_9
1	−1	−1	−1	−1	−1	9.8	7.5	12.5	41.5
26	1	−1	−1	−1	1	30.2	35.0	22.5	45.0
5	−1	1	−1	−1	1	17.5	17.5	12.5	20.0
22	1	1	−1	−1	−1	12.5	10.0	7.5	12.5
3	−1	−1	1	−1	1	512.5	1950.0	2070.0	3030.0
24	1	−1	1	−1	−1	655.0	670.0	450.0	1700.0
7	−1	1	1	−1	−1	342.5	262.5	410.0	322.5
20	1	1	1	−1	1	1020.0	1050.0	970.0	1230.0
2	−1	−1	−1	1	1	82.5	145.0	162.5	145.0
25	1	−1	−1	1	−1	19.0	22.0	17.5	25.0
6	−1	1	−1	1	−1	9.3	5.8	5.0	12.5
21	1	1	−1	1	1	27.5	22.5	15.0	20.0
4	−1	−1	1	1	−1	270.0	237.5	337.5	717.5
23	1	−1	1	1	1	282.5	710.0	650.0	547.5
8	−1	1	1	1	1	172.5	237.5	210.0	190.0
19	1	1	1	1	−1	172.5	155.0	257.5	435.0
9	−1	0	0	0	0	45.8	52.5	62.5	57.5
27	1	0	0	0	0	77.5	62.5	70.0	113.5
11	0	−1	0	0	0	195.8	262.5	252.5	276.3
12	0	1	0	0	0	33.0	22.5	15.0	27.5
13	0	0	−1	0	0	20.0	15.0	17.5	17.5
14	0	0	1	0	0	337.5	117.5	105.0	177.5
15	0	0	0	−1	0	70.0	147.5	60.0	147.5
16	0	0	0	1	0	83.8	62.5	132.5	105.0
17	0	0	0	0	−2	40.0	40.0	22.5	60.0
18	0	0	0	0	2	287.5	450.0	482.5	495.0
10	0	0	0	0	0	67.5	77.5	45.0	107.5

Table 3—Estimated coefficients of four second order fitted models, rounded to integer values

Est. coeff.	$\hat{y}_0$	$\hat{y}_3$	$\hat{y}_6$	$\hat{y}_9$
b_0	74	35	25	18
b_1	46	− 10	− 46	− 23
b_2	− 14	−125*	−115*	−237*
b_3	196*	284*	288*	445*
b_4	− 86*	−142*	−124*	−242*
b_5	48*	151*	147*	118
b_{11}	− 14	29	44	82
b_{22}	39	114	113	148
b_{33}	104	38	39	94
b_{44}	2	77	74	122
b_{55}	22	53	57	66
b_{12}	36	101	128*	173*
b_{13}	54*	− 1	− 36	− 14
b_{14}	− 54*	47	80	24
b_{15}	22	− 55	− 48	−164*
b_{23}	4	−107*	− 93	−227*
b_{24}	− 29	39	30	153
b_{25}	47	− 63	− 98	− 38
b_{34}	−106*	−170*	−162*	−280*
b_{35}	28	153*	142*	105
b_{45}	− 29	− 88	−111	−159

* Significant at $\alpha = 0.05$ or at a smaller α-level.

(a) Only terms in x_2, x_3, and x_5 appear to be needed for responses y_6 and y_9.

(b) Only terms in x_2, x_3, x_5, and x_3x_4 appear to be needed for y_0 and y_3.

When we actually refit the models $\ln \hat{y} = b_0 + b_2 x_2 + b_3 x_3 + b_5 x_5 + b_{34} x_3 x_4$, the term b_{34} becomes nonsignificant only for y_9 so, for uniformity, we reproduce the fitted equations with b_{34} present in all of them in Table 4.

Examination of the estimated coefficients in Table 4 reveals a remarkable similarity over time, and clearly calls for further conjecture and checking. An obvious question is whether or not one model would provide a satisfactory fit to all 108 data points. Performing this calculation gives

$$\ln y = 4.515 - 0.484x_2 + 1.537x_3 + 0.461x_5 - 0.350x_3x_4,$$
$$(0.05) \quad (0.07) \quad (0.07) \quad (0.06) \quad (0.07)$$

with $R^2 = 0.870$, a remarkably good fit in the circumstances. The numbers in parentheses are the respective standard errors, and all coefficients are highly significant. An overall check of whether the time effect can be so omitted is provided by comparing the residual sum of squares when a single model is fitted with the total of the individual residual sums of squares from the four separate models, and computing an appropriate F-statistic. This turns out to be not significant at the $\alpha = 0.05$ level. A referee points out, however, that there is some evidence (at the $\alpha = 0.02$ level) that the constant term varies linearly with time but that none of the other coefficients show such an effect and the R^2 value is increased only to 0.876 if a varying constant term is introduced. We might interpret all this to indicate broadly that, although storage time effects may perhaps be ignored in the present context, data taken over a longer period of time might need to be rechecked for this feature.

The restriction $y_t \leqslant 1000$ translates into $\ln y_t \leqslant 6.908$. This implies that $- 0.484x_2 + 1.537x_3 + 0.461x_5 - 0.350x_3x_4 \leqslant 2.393$. Points (x_2, x_3, x_4, x_5) that obey this inequality are satisfactory ones.

Table 4—Fitted coefficients from four equations fitted to the response ln y. Numbers in parentheses are the standard errors of those coefficients

Est. coeff.	$\ln \bar{y}_0$	$\ln \bar{y}_3$	$\ln \bar{y}_6$	$\ln \bar{y}_9$
b_0	4.408 (0.08)	4.458 (0.10)	4.389 (0.12)	4.804 (0.11)
b_2	−0.267 (0.10)	−0.508 (0.12)	−0.545 (0.15)	−0.618 (0.14)
b_3	1.438 (0.10)	1.530 (0.12)	1.621 (0.15)	1.561 (0.14)
b_5	0.369 (0.09)	0.590 (0.11)	0.558 (0.13)	0.326 (0.12)
b_{34}	−0.361 (0.11)	−0.392 (0.13)	−0.346 (0.16)	−0.300 (0.15)
R^2	0.919	0.904	0.877	0.878

DISCUSSION

WHAT HAS BEEN ACHIEVED by our re-analysis? First, an enormous simplification has resulted, in that the response values are now predicted by one equation with five coefficients instead of four equations with 84 coefficients. Second, it is now seen that the response values taken at the four times have a common pattern, something not clear from the original fitting process. Third, the variable x_1 no longer appears, indicating that, over the range of values selected for the experiment, namely, 175 – 205°F, i.e., 79.4 – 96.1°C, the preheating temperature is not crucial to the resulting response values. This example shows the enormous value of considering transformations on the response variable. General methods for finding and evaluat-ing such transformations may be found in all the statistical references quoted.

REFERENCES

Box, G.E.P. and Cox, D.R. 1964. An analysis of transformations. J. Roy. Statist. Soc. B26: 211.
Box, G.E.P., Hunter, W.G. and Hunter, J.S. (1978). "Statistics for Experimenters." John Wiley and Sons, Inc., New York.
Draper, N.R. and Smith, H. 1981. "Applied Regression Analysis," 2nd ed., Section 5.3. John Wiley and Sons, Inc., New York.
Swanson, A.M., Geissler, J.J., Magnino, P.J. Jr., and Swanson, J.A. 1967. Use of statistics and computers in the selection of optimum food processing techniques. Food Technol. 21(11): 99.

to bottling resulted in a small negative effect on free anthocyanin and color stability. This may possibly have been caused by the extra handling of the syrups during the evacuation procedure. A negative effect of daylight on red color stability was significant in one cultivar, while the effect of daylight on free anthocyanin stability was not significant for any cultivars. Direct comparison between the effect of artificial light and daylight could not be made with the present experimental design.

REFERENCES

Abers, J.E. and Wrolstad, R.E. 1979. Causative factors of color deterioration in strawberry preserves during processing and storage. J. Food Sci. 44: 75.
Blom, H. and Skrede, G. 1984. Suitability of four blackcurrant cultivars for industrial syrup production. J. Sci. Food Agric. 35: 332.
Debicki-Pospisil, J., Lovric, T., Trinajstic, N., and Sabljic, A. 1983. Anthocyanin degradation in the presence of furfural and 5-hydroxymethylfurfural. J. Food Sci. 48: 411.
Fuleki, T. and Francis, F.J. 1968. Quantitative methods for anthocyanins. 2. Determination of total anthocyanin and degradation index for cranberry juice. J. Food Sci. 33: 78.
Hunter, R.S. 1975. "The Measurement of Appearances." John Wiley & Sons, New York.
Markakis, P. 1982. Stability of anthocyanins in foods. In "Anthocyanins as Food Colors," (Ed.) P. Markakis, p. 163. Academic Press, New York.
Meschter, E.L. 1953. Fruit color loss. Effects of carbohydrates and other factors on strawberry products. J. Agric. Food Chem. 1: 574.
Nebesky, E.A., Esselen, W.B. Jr., McConnell, J.E.W., and Fellers, C.R. 1949. Stability of color in fruit juices. Food Research 14: 261.

Poei-Langston, M.S. and Wrolstad, R.E. 1981. Color degradation in an ascorbic acid-anthocyanin-flavanol model system. J. Food Sci. 46: 1218.
Ribéreau-Gayon, P. 1982. The anthocyanins of grapes and wines. In "Anthocyanins as Food Colors," (Ed.) P. Markakis, p. 209. Academic Press, New York.
Simard, R.E., Bourzeix, M., and Heredia, N. 1982. Factors influencing color degradation in blueberry juice. Lebensm.-Wiss. u.-Technol. 15: 177.
Sistrunk, W.A. and Gascoigne, H.L. 1983. Stability of color in 'Concord' grape juice and expression of color. J. Food Sci. 48: 430.
Skrede, G., Naes, T., and Martens, M. 1983. Visual color deterioration in blackcurrant syrup predicted by different instrumental variables. J. Food Sci. 48: 1745.
Snedecor, G.W. and Cochran, W.G. 1968. "Statistical Methods." Univ. Press, Ames, Iowa.
Somers, T.C. 1971. The polymeric nature of wine pigments. Phytochemistry 10: 2175.
Spayd, S.E. and Morris, J.R. 1980. Prediction of sensory color of strawberry jam. Arkansas Farm Research 29: 12.
Spayd, S.E. and Morris, J.R. 1981a. Influence of immature fruits on strawberry jam quality and storage stability. J. Food Sci. 46: 414.
Spayd, S.E. and Morris, J.R. 1981b. Effects of immature fruit and holding on strawberry puree quality and color stability. J. Amer. Soc. Hort. Sci. 106: 211.
Wrolstad, R.E., Putnam, T.P., and Varseveld, G.W. 1970. Color quality of frozen strawberries: Effect of anthocyanin, pH, total acidity and ascorbic acid variability. J. Food Sci. 35: 448.

Prediction of Beta-Carotene Decolorization in Model System Under Static and Dynamic Conditions of Reduced Oxygen Environment

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ABSTRACT

Predictions of beta-carotene losses were made with model systems stored under conditions simulating storage of dry foodstuff powders. The mathematical model utilized kinetic data collected under static conditions of water activity and oxygen concentration and was extended for the prediction of beta-cartone retention under dynamic conditions. Results showed an excellent agreement between actual and predicted retention of beta-carotene stored under dynamic conditions once the proper correction factor was used. The correction factor was required to compensate for different geometries which affected diffusion limiting reactions.

INTRODUCTION

FOODS are very sensitive and susceptible to quality losses due to chemical instability which depends on compositional and environmental factors. Kinetic studies may be utilized to minimize these undesired change and to optimize quality and/or nutrient retention. Normally the index of deterioration chosen in these kinetic studies is the least stable component. Beta-carotene fits this role due to its high instability to environmental factors (e.g. oxygen, temperature, light). Furthermore, the degradation of beta-carotene has been reported to be associated with the development of off-flavors in dehydrated carrots and sweet potato flakes (Ayres et al., 1964; Walter et al., 1970). Thus, beta-carotene provides an excellent substance to be considered as an index of deterioration for kinetic studies.

Recently, Goldman et al. (1983) showed that the decolorization of beta-carotene dispersed on microcrystalline cellulose under reduced oxygen concentration (0 – 20.9%) and at low water activity ("dry"-0.84a_w) at 35°C, followed a chain reaction. Furthermore, beta-carotene retention curves were sigmoidal with three regions (initiation period, accelerated period and a retardation period) typical of an autocatalytical radical reaction. The sigmoidal shape was also observed at high temperatures typical for air drying of foods (Stefanovich and Karel, 1982), in dilute solutions (Alekseev et al., 1968, 1972; Budowski and Bondi, 1960) and in solid films (Finkel'stein et al., 1973, 1974).

Kinetic studies of beta-carotene degradation due to moisture/water activity affects were reported by Arya et al., (1979a, b; 1983), Chou and Breene (1972), Goldman et al., (1983), Kearsley and Rodrigues (1981) and Ramakrishnan and Francis (1979). Recently, Haralampu (1983) and Haralampu and Karel (1983) presented a mathematical/kinetic model which described the effect of water activity on the rate of beta-carotene loss in a dehydrated sweet potato system stored under isothermal conditions at 40°C. The effect of oxygen on beta-carotene decolorization was reviewed by Teixeira-Neto et al. (1981).

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The overall purpose of this investigation was to develop a mathematical/kinetic model for the prediction of beta-carotene loss under typical conditions simulating dry foodstuffs during storage. The specific objectives were as follows: (1) To utilize the kinetic data (Goldman et al., 1983) collected under static conditions of water activity and oxygen concentration for the formulation of a mathematical/kinetic model; and (2) To investigate the feasibility of utilizing the model derived under static conditions for the prediction of beta-carotene retention under dynamic conditions (continuous oxygen depletion) simulating actual storage of dehydrated foodstuffs.

MATERIALS & METHODS

Model system preparation

The model system was prepared as follows (Goldman et al., 1983): 0.4% solution of beta-carotene in methylene chloride was combined with microcrystalline cellulose (MCC). The MCC was previously dried in a vacuum oven at 75°C for 24 hr. The mixture was ground with a glass mortar and pestle to obtain a homogeneous powder. Residual methylene chloride and adsorbed air were removed under vacuum of 10^{-2} mm Hg for 2 hr. The model system was equilibrated for 2 wk to definite water activities, using the common saturated salt solution procedure. All the above steps were carried out under subdued light and in a N_2 environment.

Beta-carotene determination

Beta-carotene concentration was calculated from the pigment absorption at 451 nm in n-hexane solution. An extinction coefficient of $E_{1cm}^{1\%} = 2550$ was utilized for the quantification of retained beta-carotene in the model systems. The detailed procedure was described previously (Goldman et al., 1983).

Headspace gas composition control

The necessary conditions of N_2 and O_2 concentration in the desiccator headspace were achieved with two sensitive flowmeters controlling the flow rates of these two gases. The precise oxygen concentration was determined with a digital Oxygen Analyzer (Beckman Model 0260). In the experiment carried out in nitrogen, the latter was passed through pyrogallol trap ensuring an oxygen free atmosphere.

Kinetic studies under static conditions

Static storage experiments were carried out at 35°C (± 0.5°C) and followed this routine (Goldman et al., 1983): Accurately weighed samples (0.4g) of a model system with pre-established water activity, were placed in plastic caps. The material barely covered the cap bottoms and created a sufficiently thin layer to prevent oxygen diffusion to be the rate-controlling step. The samples were placed in desiccators and the headspace environment was adjusted to the desired oxygen and relative humidity by passing the required gas mixture through a sinterglass bubbler immersed in saturated salt solution before introduction into the desiccator. The desiccators were sealed for the storage experiments only after the appropriate environment was achieved. In the "dry" experiments the required gas mixture was passed through concentrated H_2SO_4.

The desiccator headspace was sufficiently large to ensure constant environment throughout the experiment. Furthermore, at each time the desiccator was opened to withdraw samples, it was reflushed for ca. 30 – 40 min with the O_2/N_2 gas mixture at the correct relative humidity and concentration.

The data collected under static conditions were utilized to formulate the static model.

Kinetic studies under dynamic conditions

To simulate storage conditions of foodstuffs in impermeable packages, the model system was placed in serum-type reaction vials having similar volume. The headspace environment and practically all the air retained on the powder was scavenged by the following procedure: The vials were closed with a silicon rubber septum and sealed with hand crimping seal. The center of the aluminum seal was torn off and a needle inserted into the vial through the septum. Vacuum (oil pump) was applied until the pressure within the vial reached ca. 20 mm Hg. The vacuum was cut off with a three-way stopcock, which enabled direct flushing with the desired oxygen/nitrogen concentration. Evacuation and flushing were repeated three times, yielding ultimately the desired gas concentration. The syringe-needle was withdrawn and the septa were conditioned with a RTV glue. Using various vial volumes/weights of the model system provided different ratios of beta-carotene/oxygen. A typical ratio of initial concentration was: $0.13 \ 10^{-4}$M beta-carotene/$0.54 \ 10^{-4}$M oxygen. Samples were placed on a shaker at $35°C$ in complete darkness. Samples were withdrawn periodically and analyzed in duplicate.
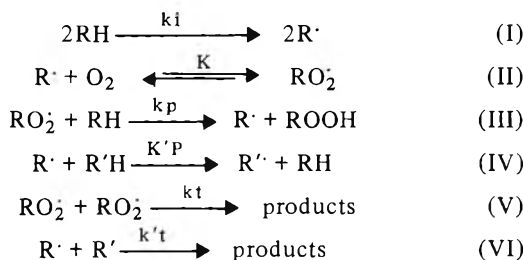
Headspace analysis

The headspace of the reaction vials was analyzed by withdrawing a gas sample with a syringe through the vial silicon rubber septum. Gases were separated and analyzed using a Packard Gas Chromatograph (Model 836) equipped with thermal-conductivity detectors. The gases were separated on a two column system in a series-across-detectors configuration. Column conditions: Porapack Q 80-100 mesh (3' x 1/8'') and molecular sieve 5A 80-100 mesh (9' x 1/16''). Columns temperatures were $25°C$; the helium carrier-gas flow rate was 20 mL/min.

Statistical analysis

Statistical analysis was carried out using a computer-aided statistical software (Dixon, 1981).

RESULTS & DISCUSSION

In accordance with the theory of free radicals chain oxidation of hydrocarbons (Emanuel et al., 1967) degradation of beta-carotenes is postulated to proceed as follows (Stefanovich and Karel, 1982):

$$2RH \xrightarrow{ki} 2R^{\cdot} \qquad (I)$$

$$R^{\cdot} + O_2 \xleftrightarrow{K} RO_2^{\cdot} \qquad (II)$$

$$RO_2^{\cdot} + RH \xrightarrow{kp} R^{\cdot} + ROOH \qquad (III)$$

$$R^{\cdot} + R'H \xrightarrow{K'P} R'^{\cdot} + RH \qquad (IV)$$

$$RO_2^{\cdot} + RO_2^{\cdot} \xrightarrow{kt} products \qquad (V)$$

$$R^{\cdot} + R'^{\cdot} \xrightarrow{k't} products \qquad (VI)$$

The rate of consumption of a hydrocarbon in a chain reaction with a second-order chain termination is (using the same terminology of Stefanovich and Karel, 1982):

$$dC/dt = B \ C \ \sqrt{bo \ Co + b(Co - C)} \qquad (1)$$

$$B = kp\sqrt{Kks \ PO_2}/\sqrt{kt} \qquad (2)$$

where: bo is the initiation rate constant of unoxidized beta-carotene; b is the initiation rate constant of the products; B is a constant derived from Eq. (2); Co is the initial beta-carotene concentration; C is the beta-carotene concentration; kp and kt are the rate constants of reaction (III) and (V); k_s is the solubility coefficient of oxygen in carotene; K is the equilibrium constant of reaction II; PO_2 is the partial pressure of oxygen; and t is time.

Integration of Eq. (1), and using the dimensionless variables suggested by Gagarina et al. (1970), yields:

$$\ln[(1+\sqrt{1-C/Co})/(1-\sqrt{1-C/Co})] = a\sqrt{b-bo} \ \sqrt{Co} \ t \qquad (3)$$

where a is a constant. Denoting all the constants in the right-hand side of Eq. (3) as the effective rate constant, $\sigma \ (=a\sqrt{b-bo} \ \sqrt{Co})$, gave the following equation:

$$\ln \ [1 + \sqrt{1 - C/Co}]/(1 - \sqrt{1 - C/Co})] = \sigma \ t \qquad (4)$$

Eq. (4) may be used to fit the experimental data of any one initial concentration. Fig. 1 depicts the linear relationship obtained using the coordinates of Equation (4) for beta-carotene decolorization under static conditions of oxygen and water activity in the headspace. It can be seen that the experimental results for storage of up to 60 days are in good agreement with Eq. (4). Hence, the effective rate constant, σ may be derived from the slope, $\ln[(1 + \sqrt{1-C/Co})/ (1 - \sqrt{1 - C/CO})]$ vs. t. Following this procedure the effective rate constants for "dry" conditions and different oxygen content in the environment headspace were calculated as summarized in Table 1. The data showed that reducing oxygen concentration from 20.9% (air) to 1% resulted in a decrease of approximately one order of magnitude in the effective decolorization rate.

The effect of water activity at a constant 2% oxygen concentration on the effective rate constant is given in Table 2. It can be seen that the increase in water activity from "dry" to 0.84 resulted in a 1.5 fold decrease of the decolorization rate constant.

The dependency of the decolorization rate on the initial concentration of beta-carotene impregnated on MCC was studied under 2% and 20.9% oxygen (Fig. 2, 3, and 4).
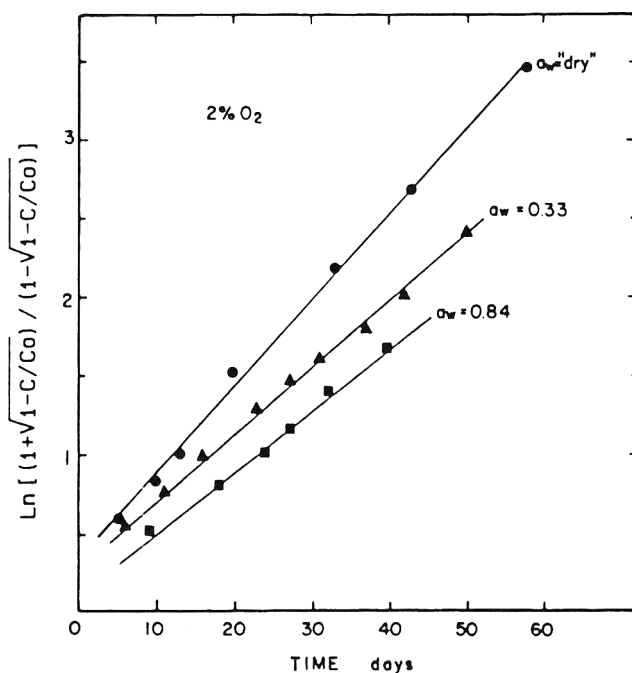

Fig. 1—Transformation of the kinetic curves of beta-carotene decolorization under different a_W and at 2.0% oxygen (Co = 2.53, 1.80 and 2.27 ms/s dry powder at "dry," 0.33 and 0.88 a_W, respectively). Data adopted from Goldman et al. (1983).

Table 1—Effective rate constant (σ) for the degradation of beta-carotene under various static oxygen concentrations (a_W = "dry" conditions @ $35°C$)

Oxygen, %	20.9	15.0	10.0	5.0	2.0	1.0
Effective rate constant, day^{-1}	0.366	0.286	0.206	0.167	0.059	0.043
Correlation coefficient, r^2	0.995	0.998	0.997	0.997	0.996	0.995

Budowski and Bondi (1960) and Gagarina et al. (1970) found that the time for complete consumption of the hydrocarbon was shorter the higher its initial concentration. Hence, they reported that beta-carotene stability was improved in dilute solutions compared to concentrated solutions. However, our results (Fig. 3 and 4) indicated that for beta-carotene concentration typical of dehydrated foods (e.g., 2.11-3.87 mg/g dry powder), decolorization rates were similar. Only at a low beta-carotene concentration (i.e., 0.63 mg/g dry powder) an increase in the decolorization rate was observed. The decrease in the effective rate constant to a certain value where it becomes independent of the initial concentration was reported previously (Stefanovich and Karel, 1982) and may be related to oxidation which occurred at a maximum rate at the monolayer value. As only the top layer would oxidize at the maximum rate, the rest is limited by oxygen diffusion, thus having an overall slower rate of oxidation. Hence, only at low Co did the initial beta-carotene concentration (below ∿ 1 mg/g dry powder) have an apparent significant effect on decolorization rates.

To elucidate the effect of oxygen and water activity on beta-carotene decolorization, a mathematical model was sought. Based on the data of Tables 1 and 2 the following relationships were determined:

oxygen effect ($1.0 \leqslant PO_2 \leqslant 20.9\%$ @ "dry" conditions)

$$\sigma = 2.982*10^{-2} + 1.656*10^{-2}\ PO_2 \qquad r^2 = 0.996 \quad (5)$$

water activity effect ("dry" $\leqslant a_w \leqslant 0.84$ @ $PO_2 = 2.0\%$)

$$\sigma = 0.056\ \exp\ (-0.4833\ a_w) \qquad r^2 = 0.953 \qquad (6)$$

where r is the correlation coefficient.

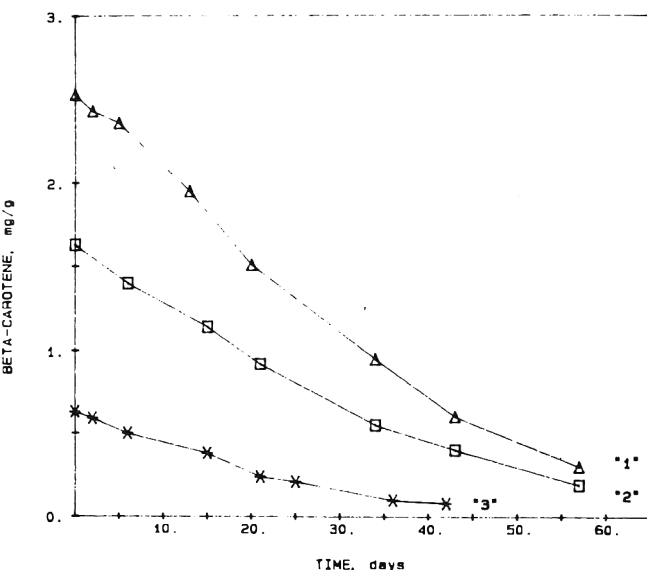Finkel'stein et al. (1974) reported on linear proportional effect of oxygen on the oxidation rate of beta-carotene at $35°C$ and above 150 mm Hg oxygen. Our data support their findings and extend the linear relationship from 20.9% to a very low oxygen concentration.

The general shape of beta-carotene decolorization effective rate constant (Eq. 6) expressing the effect of water activity is similar to the behavior presented by Haralampu and Karel (1983), but the models are not identical. This is not surprising as empirical models were established in both cases.

To account simultaneously for both oxygen and water activity, Eq. (5) and (6) were combined and incorporated in the following model:

$$\sigma = f\ (PO_2)\ \exp[\ f(a_w)\ ] \qquad (7)$$

using this relationship the model established was:

$$\sigma = [2.982*10^{-2} + 1.656*10^{-2}\ PO_2]\exp(-0.4833 a_w) \quad (8)$$

It is worth noting that the model (Eq. 8) which describes the relationship between the effective decolorization rate constant as a function of water activity and oxygen is empirical, and hence should be used with care. Nevertheless, the data utilized for the establishment of the kinetic model was based on the theory of free radical reaction. Furthermore, as we have constrained our experiments to a narrow range of oxygen and water activity, it is expected that prediction based on the derived model would normally be quite limited.

To demonstrate the applicability of the model established under static environment to conditions pertinent to actual storage of food powders, tests were carried out under dynamic conditions. For this purpose decolorization was followed under conditions where one of the environmental factors (i.e., oxygen) changed continuously. These condi-
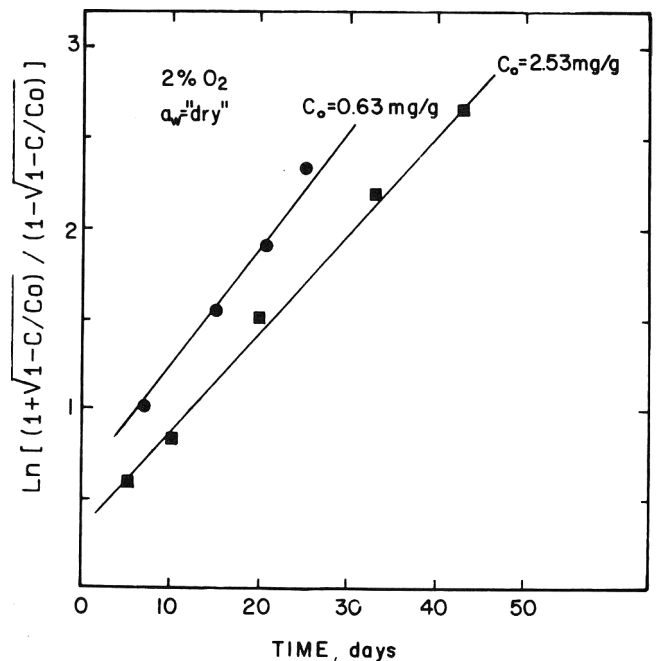

Fig. 2—Decolorization of beta-caroene at a_w = "dry" @ 2.0% oxygen. Initial beta-carotene concentration: "1" — 2.53; "2" — 1.63; "3" — 0.63 mg/g dry powder.

Fig. 3—The effect of various initial concentration, Co, on beta-carotene decolorization (stroage conditions: 2.0% oxygen @ a_w = "dry").

Table 2—Effective rate constant (σ) for the degradation of beta-carotene under various water activities (2% oxygen @ 35°C)

Water activity	"dry"	0.11	0.33	0.52	0.84
Effective rate constant, day^{-1}	0.059	0.053	0.045	0.044	0.038
Correlation coefficient, r^2	0.998	0.995	0.982	0.997	0.995

tions of constant water activity and depleting oxygen concentration are similar to storage of dehydrated foodstuffs in impermeable packages where oxygen is depleted due to oxidation reactions. Beta-carotene retention was higher in dynamic conditions when compared with static tests (Fig. 4 and 5).

A computer program was written to simulate these dynamic conditions and to account for beta-carotene retention. Having the model for the effective rate constant as a function of water activity and oxygen the prediction numerical model utilized the experimental values of time and oxygen concentration for the calculation of beta-carotene retention at any given time.

Dividing the time into n small intervals (yielding dt $\sim$ 0.2 days), the effective rate constant was expressed as:

$$\sigma = f[PO_2(t_i)] \exp \left\{ f[a_w(t_i)] \right\} \qquad (9)$$

using this expression, Eq. (4) was rewritten:

$$(1 + \sqrt{1 - C/Co})/(1 - \sqrt{1 - C/CO}) = \exp \left(\sum_{i=1}^{n} \sigma_i \, dt \right) \qquad (10)$$
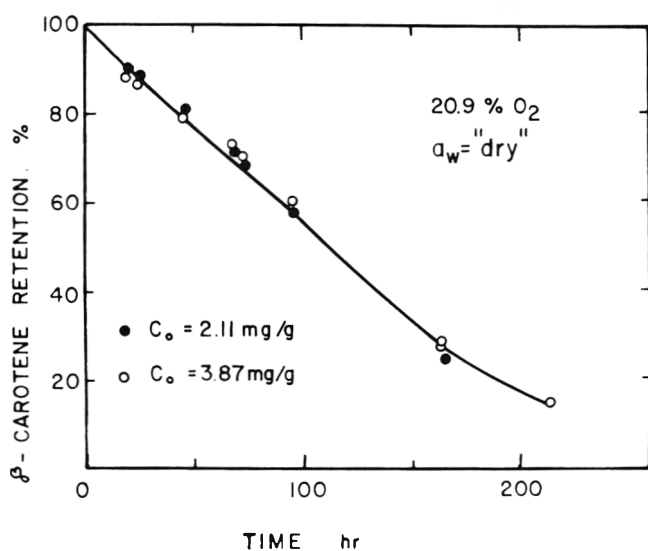

Fig. 4—Effect of initial concentration, Co, on beta-carotene degradation stored in air and "dry" conditions.

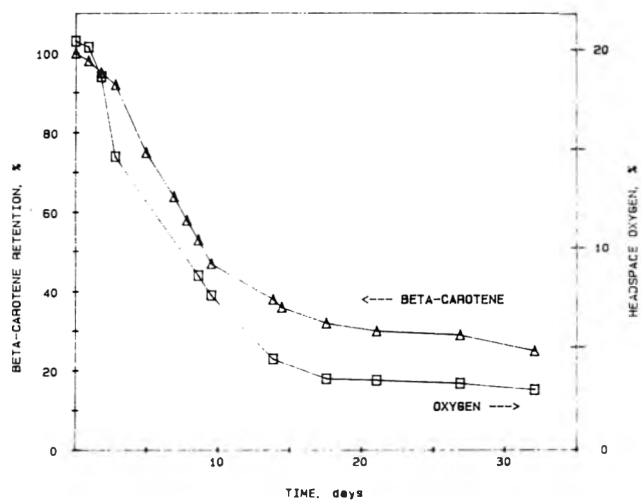*Fig. 5—Beta-carotene decolorization under dynamic storage conditions (Co = 2.75 mg/g dry powder; initial oxygen 20.9%; a_w = "dry").*

Or by rearrangement the concentration at any given time may be derived:

$$C = Co \left\{ 1 - [(E-1)/(E+1)]^2 \right\} \qquad (11)$$

where $E = \epsilon xp \left(\sum_{i=1}^{n} \sigma_i \, dt \right)$

Linear interpolation was used to calculate the oxygen values for each time interval. The results obtained showed a discrepancy between actual and predicted values. Since the discrepancy was constant, a correction factor, fc, was proposed. The correction factor can be explained by the differences between the dynamic and static tests due to the physical differences mainly related to oxygen diffusivity. Hence, the static tests were designed to avoid limitation in oxygen supply, as in the dynamic tests. Moreover, in the dynamic tests, the ratio between the void headspace and the amount of material filling the vial had an influence on the concentration of the available oxygen. Thus, the need for an experimental correction factor expressing these differences is not surprising.

Applying an optimization technique (the simplex method; NAG, 1977) to minimize the least-squares between observed and predicted values, the correction factor was derived. Results obtained with this procedure (Fig. 6) showed an excellent agreement between actual and predicted beta-carotene stored under dynamic conditions, using the proper correction factor, fc. Different geometries (ratio of headspace and void volume to material volume) yielded different correction factors, as can be seen from Fig. 6 for the model system stored under air. Hence, the correction factor is unique for each system and expresses the deviation from ideal conditions under which kinetic studies are normally carried out.

The existence of a correction factor showed that in actual food storage the geometry of the package is important since it controls the diffusion limited reactions. Thus, this approach of establishing the model may be utilized in other systems, providing that the proper correction factor was calculated to express the experimental conditions pertinent for each specific system. However, the establishment of a correction factor may be difficult in some systems because of unknown interrelationships which can affect results. Therefore, all aspects of a system should be thoroughly investigated before a correction factor is established and utilized.

In conclusion, the mathematical/kinetic model for the prediction of beta-carotene loss under typical conditions simulating dry foodstuff powders during storage was estab-
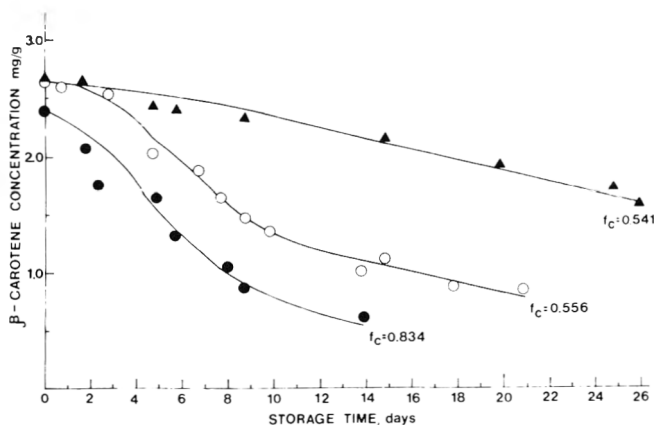

Fig. 6—Comparison between observed and predicted beta-carotene retention under dynamic storage conditions (initial oxygen concentration: ▲, 5%; ●, ○ – 20.9%; predicted values expressed by full line).

lished under static conditions and utilized for prediction under dynamic conditions. The latter simulated actual storage of dehydrated food powders. The correction factor was required to compensate for several constraints which may be affected due to diffusion limiting reactions. The correction factor was unique for each system and expressed the deviation from ideal conditions under which kinetic studies are normally carried out.

REFERENCES

Alekseev, E.V., Gagarina, A.B., Evteeva, N.M., Vakulova, L.A., Samokhalov, G.I., and Emanuel, N.M. 1968. Kinetic principles of the oxidation of polyenic hydrocarbons. Communication 1. Decomposition of beta-carotene in the presence of free radical initiators. Izv. Akad. Nauk SSSR, Ser. Khim. 11: 2474.

Alekseev, E.V., Gagarina, A.B., Vakulova, L.A., Samokhalov, G.I. Rozantsev, E., and Emanuel, N.M. 1972. Inhibition of beta-carotene oxidation in an aromatic solvent. Izv. Akad. Nauk SSSR, Ser. Khim. 312.

Arya, S.S., Natesan, V., Parihar, D.B., and Vijayarghavan, P.K. 1979a. Stability of beta-carotene in isolated systems. J. Food Technol. 14: 571.

Arya, S.S., Natesan, V., Parihar, D.B., and Vijayarghavan, P.K. 1979b. Stability of carotenoids in dehydrated carrots. J. Food Technol. 14: 579.

Arya, S.S., Natesan, V., and Vijayarghavan, P.K. 1983. Stability of carotenoids in freeze-dried papaya (Carica papaya). J. Food Technol. 14: 571.

Ayres, J.E., Fishwick, M.J., Land, D.G., and Swain, T. 1964. Nature (Lond.) 203: 81.

Budowski, P. and Bondi, A. 1960. Autoxidation of carotene and vitamin A. Influence of fat and antioxidants. Arch. Biochem. Biophys. 89: 66.

Chou, H. and Breene, W. 1972. Oxidative decolorization of beta-carotene in low-moisture model systems. J. Food Sci. 37: 66.

Dixon, W.J. 1981. 'BMDP-Statistical Software', University of California Press, Berkeley, CA.

Emanuel, N.M., Denisov, E.T., and Maizus, Z.K. 1967. "Liquid Phase Oxidation of Hydrocarbons," p. 9. Plenum Press, New York, N.Y.

Finkel'stein, E.I., Alexeev, E.V., and Kozlov, E.I. 1973. Kinetics of the autoxidation of solid films of beta-carotene. Dokl. Akad. Nauk SSSR 208(6): 1408.

Finkel'stein, E.I., Alexeev, E.V., and Kozlov, E.I. 1974. Kinetics relationships of the solid state autoxidation of beta-carotene. Zhurnal Organicheskoi Khimii. 10(5): 1027.

Gagarina, A.B., Kasaikina, O.T., and Emanuel, N.M. 1970. Kinetics of autooxidation of polyene hydrocarbons. Dokl. Akad. Nauk SSSR 195(2): 387.

Goldman, M., Horev, B., and Saguy, I. 1983. Decolorization of beta-carotene in model systems simuating dehydrated foods. Mechanism and kinetic principles. J. Food Sci. 48: 751.

Haralampu, S.G. 1983. The development of a dynamic stability test for food, Ph. D. thesis, Massachusetts Institute of Technology, Cambridge, MA.

Haralampu, S.G. and Karel, M. 1983. Kinetic models for moisture dependence of ascorbic acid and beta-carotene degradation in dehydrated sweet potato. J. Food Sci. 48: 1776.

Kearsley, M.W. and Rodrigues, N. 1981. The stability and use of natural colours in foods: anthocyanins, beta-carotene and riboflavin. J. Food Technol. 16: 421.

NAG (Numerical Algorithms Group). 1977. "NAG FORTRAN Library (Program E04CCF)." Oxford, UK.

Ramakrishnan, T. and Francis, F.J. 1979. Stability of carotenoids in model aqueous systems. J. Food Quality 2: 177.

Stefanovich, A.F. and Karel, M. 1982. Kinetics of beta-carotene degradation at temperatures typical of air drying of foods. J. Food Process. & Preserv. 6: 227.

Teixeira-Neto, R.O., Karel, M., Saguy, I., and Mizrahi, S. 1981. Oxygen uptake and beta-carotene decolorization in dehydrated food model. J. Food Sci. 46: 665.

Walter, W.M. Jr., Purcell, A.E., and Cobb, W.Y. 1970. Fragmentation of beta-carotene in autoxidating dehydrated sweet potato flakes. J. Agric. Food Chem. 18: 881.

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however, no such effects could be observed in this storage experiment. A few explanations may be proposed as to why no antioxidative effect was observed in the herring mince: (1) fatty fish oxidizes very quickly, and the additions of MRP occurred too late, (2) the levels of MRP used were too low, and (3) the antioxidative MRP were inactivated by components in the herring mince.

REFERENCES

Ackman, R.G. 1980. In "Advances in Fish Science and Technology," (Ed.) J.J. Connell, p. 86. Fishing News Books Ltd., Farnham, U.K.

AOAC. 1970. "Official Methods of Analysis," 11th ed. Association of Official Analytical Chemists, Washington, DC.

Bilinski, E., Jonas, R.E.E. and Lau, Y.D. 1979. Control of rancidity in frozen Pacific herring, Clupea harengus pallasi: Use of sodium erythorbate. J. Fish. Res. Board Can. 36: 219.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total extraction and purification. Can J. Biochem. Physiol. 37: 911.

Davies, O.L. and Goldsmith, P.L. 1972. "Statistical Methods in Research and Production," Longmans Group Limited, London.

Deng, J.C., Matthews, F.R., and Watson, C.M. 1977. Effect of chemical and physical treatments on rancidity development of frozen mullet (Mugil cephalus) fillets. J. Food Sci. 42: 344.

Grosch, W. 1982. Lipid degradation products and flavour. In "Food Flavours. Part A. Introduction," (Ed.) I.D. Morton, and A.J. MacLeod, p. 325. Elsevier Publishing Company, Amsterdam.

Hall, G. and Lingnert, H. 1984. Flavor changes in whole milk powder during storage. 1. Odor and flavor profiles of dry milk with additions of antioxidants and stored under air and nitrogen. J. Food Quality. 7(2): 131.

Hardy, R. and Smith, I.G.M. 1976. The storage of mackerel (Somber scombrus). Development of histamine and rancidity. J. Sci. Food Agric. 27: 595.

Hiremath, G.G. 1973. Prevention of rancidity in frozen fatty fishes during cold storage. Indian Food Packer 27(6): 20.

Lingnert, H. 1980. Antioxidative Maillard reaction products. 3. Application in cookies. J. Food Proc. Preserv. 4: 219.

Lingnert, H. and Eriksson, C.E. 1981. Antioxidative effect of Maillard reaction products. Prog. Food Nutr. Sci. 5: 453.

Lingnert, H., Eriksson, C.E. and Waller, G.R. 1983. Characterization of antioxidative Maillard reacton products from histidine and glucose. ACS Symp. Ser. 215: 335.

Lingnert, H. and Lundgren, B. 1980. Antioxidative effect of Maillard reaction products. IV. Application in sausage. J. Food Proc. Preserv. 4: 235.

Moledina, K.H., Regenstein, J.M., Baker, R.C., and Steinkraus, K.H. 1977. Effects of antioxidants and chelators on the stability of frozen stored mechanically deboned flounder meat from racks after filleting. J. Food Sci. 42: 759.

Smith, J.G.M., McGill, A.S., Thomson, A.B. and Hardy, R. 1980. In "Advances in Fish Science and Technology," (Ed.) J.J. Connell, p. 303. Fishing News Books Ltd., Farnham, U.K.

von Sydow, E., Andersson, J., Anjou, K., Karlsson, G., Land, D., and Griffiths, N. 1970. Aroma of bilberries (Vaccinium myrtillus). 2. Evaluation of the press juice by sensory methods and by gas chromatography and mass spectrometry. Lebensm. Wiss. Technol. 3: 11.

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A Research Note
Effect of Phosphates on Bacterial Growth in Refrigerated Uncooked Bratwurst

R. A. MOLINS, A. A. KRAFT, and D. G. OLSON

──────────── ABSTRACT ────────────

The effects of 0.5% sodium acid pyrophosphate (SAPP), sodium tripolyphosphate (STPP), tetrasodium pyrophosphate (TSPP) and sodium polyphosphate glassy (SPG) on aerobic mesophilic and psychrotrophic bacterial growth and on survival of inoculated *Staphylococcus aureus* Z 88 were investigated in uncooked bratwurst stored at 5°C for 7 days. No significant microbial inhibition by phosphates was found, although SAPP addition resulted in consistently lower total aerobic plate counts. Phosphate-induced pH differences in the sausages had no effect on bacterial numbers. The possible role of meat enzymes in the hydrolysis of condensed phosphates to microbiologically inactive species is discussed.

INTRODUCTION

ANTIMICROBIAL EFFECTS of phosphates in poultry products was well documented in earlier work of Spencer and Smith (1962), Steinhauer and Banwart (1963), and Chen et al. (1973) who demonstrated that dipping chicken parts or carcasses in phosphate-containing chilling water resulted in decreased bacterial counts and prolonged shelf life. Bacterial growth inhibition in laboratory media has been attributed to phosphates by Post et al. (1963), Elliott et al. (1964), Gray and Wilkinson (1965) and more recently by Snyder and Maxcy (1979), Firstenberg-Eden et al. (1981) and Seward et al. (1982). In this laboratory 0.5% tetrasodium pyrophosphate (TSPP) was lethal or highly inhibitory to *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium* and two commercial lactic starter cultures in laboratory media. Unheated sodium tripolyphosphate (STPP) and sodium polyphosphate glassy (SPG) were not as inhibitory and lost their antimicrobial properties when heated to 100+ °C. Unheated sodium acid pyrophosphate (SAPP) was only slightly inhibitory to the microorganisms tested and enhanced rather than decreased recoveries in the heated form. Furthermore, in most experiments, 3-hr old cultures and the Gram positive organisms used were more sensitive to phosphates than were older cultures (24-hr) and Gram negative bacteria (Molins et al., 1984).

Renewed interest in the applicability of phosphates as bactericidal or bacteriostatic compounds was raised by their approval as additives for use in a wide variety of cooked meat products by the USDA (1982). Although the physical and chemical contributions of phosphates to meat product quality with regard to texture, improved water holding capacity and binding, increased emulsion stability and other factors reviewed by Knipe (1982) have been determinant in their use and approval, only limited attention has been paid to the potential antimicrobial application of phosphates to meats and meat products. Ivey and Robach (1978) found SPG and SAPP to have no significant effects on the growth and toxin production by *Clostridium botulinum* when used alone or combined with nitrite in canned comminuted pork, but to have synergistic effects

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when sorbic acid was present. These findings were later confirmed with SAPP by Roberts et al. (1981), Wagner and Busta (1983), and Nelson et al. (1983). Nielsen and Zeuthen (1983) reported that 0.3% STPP alone had little influence on the growth of *Brochothrix thermosphacta* or *Serratia liquefaciens* in sliced, vacuum packed bologna under refrigerated storage (2°C), but that an acid mixture of SAPP, STPP and SPG markedly inhibited the growth of both spoilage microorganisms under the same conditions.

The present work was undertaken to determine effects of the highest legally allowed level (0.5%) of two food-grade poly- and two pyrophosphates commonly used in the meat industry on the survival of *S. aureus* and on total mesophilic and psychrotrophic bacterial counts in refrigerated, uncooked bratwurst-type sausages. Although the use of phosphates in uncooked meat products is not permitted, bratwurst was selected for the present phase of a series of studies since this product is unique insofar as it is marketed in uncooked as well as cooked, vacuuum packed forms and usually does not include nitrite in its formulation.

MATERIALS & METHODS

BRATWURST was prepared from coarse ground pork butts (20% fat) obtained in frozen, 12-lb packages from the Iowa State Meat Laboratory. The meat was allowed to thaw 48 hr at 2 - 4°C and the drip was reincorporated into the meat by blending with a Hobart C-10 mixer (Hobart Mfg. Co., Troy, OH) for 2 min at low speed. Bratwurst spice mix (2%, w/w) (Saratoga Specialties, Elmhurst, IL) was similarly blended, providing a concentration of 1.7% NaCl. Portions weighing 1000g received the appropriate volume of a 10% aqueous solution (w/v) of one of the test phosphates to attain a level of 0.5% phosphate in the final product. After further blending, each 1000-g portion of phosphate-containing sausage mix was subdivided. One-half was inoculated with a diluted, 24-hr culture of *S. aureus* Z 88 (Food Technology Dept., ISU) grown in Brain Heart Infusion (BHI) broth (BBL) so as to number ca. 10^4 cells/g while the other half received an equivalent amount of sterile distilled water. The final blending was followed by stuffing into 1-inch dia., edible collagen casings (Devro, Inc., Somerville, NJ). All sausages were packaged in polyethylene bags, twist-tied and stored in a display case at about 5°C. Inoculated and uninoculated controls were prepared with and without phosphates. Samples were taken on days 0, 1, 3, 5 and 7 for microbiological examination. Thirty grams of sausage were weighed, blended with 270 ml of 0.1% peptone water, diluted and plated following standard methods. The numbers of surviving *S. aureus* were determined from inoculated samples (Baird-Parker medium, BBL, 24 hr at 37°C) and total mesophilic and psychrotrophic counts (Trypticase soy agar, TSA, BBL, 30°C and 5°C, respectively) were obtained from uninoculated sausages. The experiments were replicated four times and after logarithmic transformation the data were analyzed by using an SAS computer program with ANOVA and GLM procedures.

RESULTS & DISCUSSION

NONE OF THE PHOSPHATES tested proved to have a significant ($P < 0.05$) effect on the survival of inoculated *S. aureus*. *S. aureus* decreased in numbers by less than one log cycle throughout the 7-day storage period at 5°C for all samples including controls. This result is in sharp contrast with the lethality exhibited by 0.5% unheated TSPP, STPP or SPG on *S. aureus* when these phosphates were
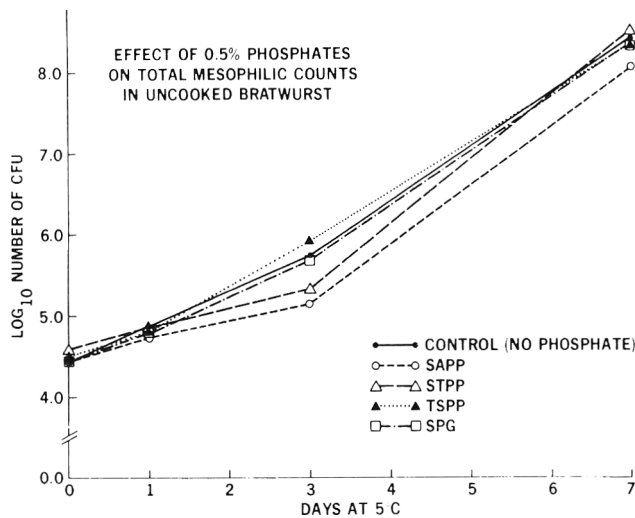
Fig. 1—Effect of 0.5% phosphates on total mesophilic counts in uncooked bratwurst.
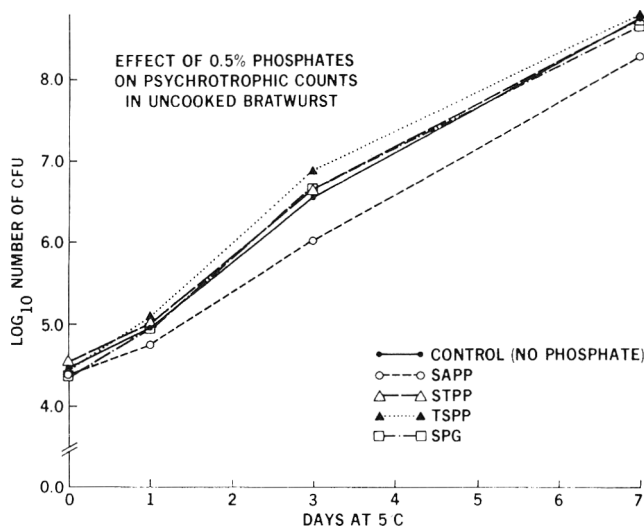

Fig. 2—Effect of 0.5% phosphates on psychrotrophic counts in uncooked bratwurst.

present in a laboratory medium in previous work (Molins et al., 1984).

The growth of mesophilic (Fig. 1) and psychrotrophic (Fig. 2) bacteria was not significantly (P < 0.05) reduced by any phosphate. However, SAPP and STPP caused a slight lengthening of the lag phase of growth of mesophiles. SAPP also resulted in consistently lower numbers of aerobic mesophilic and psychrotrophic bacteria than any other phosphate or controls throughout the experimental period, but these effects were not statistically significant (P < 0.05).

The presence of the enzymes, poly- and pyrophosphatases, in muscle cells may account for the loss of the inhibitory properties that condensed phosphates have on bacterial growth in systems other than meat, where no such enzymes are present. Rapid hydrolysis of poly- and pyrophosphates to the ortho forms and the important roles of pH, temperature, time and inactivation of phosphate-hydrolyzing enzymes by cooking temperatures on the retention of the active, unhydrolyzed forms of phosphates was demonstrated by Awad (1968). With ground or comminuted meat products and systems, additional release of phosphatases by muscle cell rupture during grinding may further increase the rate of phosphate hydrolysis. Other

complex interactions, such as those between phosphates and meat proteins (Nikkila et al., 1967; Newbold and Tume, 1981), may also explain the failure of phosphates as bacterial inhibitors in uncooked, processed meats.

Marked pH differences between bratwurst containing different phsophates existed throughout the 7-day storage period at $5°C$ without corresponding variations in bacterial numbers. Also, pH differences between treatments decreased only slightly after 1 wk of storage when final bacterial counts were in excess of 10^8 CFU/gram.

Additional research is justified on possible effects of immediate cooking after formulation for retention of the microbial inhibitory properties of phosphates in meat products.

REFERENCES

Awad, M.K. 1968. Hydrolysis of polyphosphates added to meat. M.S. thesis, The Univ. of Alberta, Edmonton, Alberta.
Chen, T.C., Culotta, J.T., and Wang, W.S. 1973. Effect of water and microwave energy precooking on microbiological quality of chicken parts. J. Food Sci. 38: 155.
Elliott, R.P., Straka, R.P., and Garibaldi, J.A. 1964. Polyphosphate inhibition of growth of pseudomonads from poultry meat. Appl. Microbiol. 12: 517.
Firstenberg-Eden, R., Rowley, D.B., and Shattuck, G.E. 1981. Inhibition of Moraxella-Actinetobacter cells by sodium phosphates and sodium chloride. J. Food Sci. 46: 579.
Gray, G.W. and Wilkinson, S.G. 1965. The action of ethylenediamine-tetra-acetic acid on Pseudomonas aeruginosa. J. Appl. Bacteriol. 28: 153.
Ivey, F.J. and Robach, M.C. 1978. Effect of sorbic acid and sodium nitrite on Clostridum botulinum outgrowth and toxin production in canned comminuted pork. J. Food Sci. 43: 1782.
Knipe, C.L. 1982. Effects of inorganic polyphosphates on reduced sodium and conventional meat emulsion characteristics. Ph.D. dissertation, Iowa State Univ., Ames, IA.
Molins, R.A., Kraft, A.A., Olson, D.G., and Hotchkiss, D.K. 1984. Recovery of selected bacteria in media containing 0.5% food grade poly- and pyrophosphates. J. Food Sci. 49: 948.
Nelson, K.A., Busta, F.F., Sofos, J.N., and Wagner, M.K. 1983. Effect of polyphosphates in combination with nitrite-sorbate or sorbate on Clostridium botulinum growth and toxin production in chicken frankfurters emulsions. J. Food Prot. 46: 846.
Newbold, R.P. and Tume, R.K. 1981. Comparison of the effects of added orthophosphate on calcium uptake and release by bovine and rabbit muscle sarcoplasmic reticulum. J. Food Sci. 46: 1327.
Nielsen, H.J.S. and Zeuthen, P. 1983. Influence of phosphate and glucose addition on some important spoilage bacteria in vacuum packed bologna-type sausage. J. Food Prot. 46: 1078.
Nikkila, O.E., Kuusi, T., and Kytokangas, R. 1967. Changes in fish proteins caused by storage in saline solution and their inhibition by phosphates. Study of the reactionby tracer technique. J. Food Sci. 32: 686.
Post, F.J., Krishnamurty, G.B., and Flanagan, M.D. 1963. Influence of sodium hexametaphosphate on selected bacteria. Appl. Microbiol. 11: 430.
Roberts, T.A., Gibson, A.M., and Robinson, A. 1981. Factors controlling the growth of Clostridium botulinum types A and B in pasteurized, cured meats. 2. Growth in pork slurries prepared from "high" pH meat (range 6.3 – 6.8). J. Food Technol. 16: 267.
Seward, R.A., Deibel, R.H., and Lindsay, R.C. 1982. Effects of potassium sorbate and other antiobotulinal agents on germination and outgrowth of Clostridium botulinum type E spores in microcultures. Appl. Environ. Microbiol. 44: 1212.
Snyder, L.D. and Maxcy, R.B. 1979. Effect of a_w of meat products on growth of radiation resistant Moraxella-Acinetobacter. J. Food Sci. 44: 33.
Spencer, J.V. and Smith, L.E. 1962. The effect of chilling chicken fryers in a solution of polyphosphates upon moisture uptake, microbial spoilage, tenderness, juiciness and flavor. Poultry Sci. 41: 1685.
Steinhauer, J.E. and Banwart, G.J. 1963. The effect of food grade polyphosphates on the microbial population of chicken meat. Poultry Sci. 43: 618.
USDA. 1982. Meat and poultry products: phosphates and sodium hydroxide. Fed. Register 47: 10779.
Wagner, M.K. and Busta, F.F. 1983. Effect of sodium acid pyrophosphate in combination with sodium nitrite or sodium nitrite/potassium sorbate on Clostridium botulinum growth and toxin production in beef/pork frankfurter emulsions. J. Food Sci. 48: 990.
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A Research Note

Determination of the Number and Heat Stability of α-Amylase Inhibitors in White and Red Kidney Bean (*Phaseolus vulgaris*)

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─────────── ABSTRACT ───────────

The α-amylase inhibitors from white and red kidney bean were isolated using a purification procedure that did not include a heat step. Phenyl-Sepharose chromatography of extracts from white kidney bean resolved two inhibitors. One of the inhibitors was much less stable than the other to 70°C heat treatment. Red kidney beans contained only one α-amylase inhibitor.

INTRODUCTION

MANY EDIBLE PLANTS contain substances which inhibit enzyme action (Marshall, 1975). Most of these compounds are proteins which specifically inhibit enzymes by forming complexes which block the active site or alter enzyme conformation, thus reducing their catalytic functions (Richardson, 1977). In contrast to trypsin inhibitors, inhibitors of α-amylase are not as well understood, and little is known about the structural features essential for their action (Granum, 1979; Richardson, 1980-81).

Jaffe et al. (1973) studied serveral species of legumes in which inhibitors of pancreatic and salivary α-amylase are present. The largest amount of inhibitory activity was found in cultivars of kidney beans. Marshall and Lauda (1975) and Pick and Wöber (1978) extracted an α-amylase inhibitor from white kidney bean which was active against α-amylase from *Helix pomatia*, human saliva and human pancreas. Powers and Whitaker (1977) isolated an α-amylase inhibitor from the red kidney bean which was active against human salivary, porcine pancreatic and *Tenebrio molitor* larva α-amylase but was inactive against plant and microbial α-amylases.

In each of the legumes studied, a single inhibitor of α-amylase was isolated. All previous studies utilized a heat treatment to coagulate unwanted protein as a step in the purification procedure, as the protease and α-amylase inhibitors are generally heat stable (Richardson, 1980-81). However, Frels and Rupnow (1984) purified two α-amylase inhibitors from the black bean (*Phaseolus vulgaris*) using a purification procedure which did not include a heat treatment. They postulated that the second inhibitor might be present in the other legumes studied, since one of the inhibitors they isolated was significantly more heat labile and may be destroyed during a heat treatment.

In this study, the α-amylase inhibitors from the white kidney bean and the red kidney bean (*Phaseolus vulgaris*) were purified using a modification of the methods of Frels and Rupnow (1984) to determine if multiple inhibitors were also present in these legumes.

MATERIALS & METHODS

WHITE KIDNEY BEANS (*Phaseolus vulgaris*) v. Great Northern UI-59 were obtained from the Univ. of Idaho Branch Experiment Station, Kimberly, Idaho. Red kidney beans (*Phaseolus vulgaris*) v.

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LRK California were obtained from the Univ. of Nebraska seed collection, Cooperative Extension Service Panhandle Station, Scottsbluff, NE.

Soluble starch for use as the substrate in the inhibitor assay was obtained from Mallinckrodt Inc. Porcine pancreatic α-amylase (Type 1-A, 2X crystallized, lot 43F-8085) and 3,5-dinitrosalicylic acid were obtained from Sigma Chemical Co. and phenyl-Sepharose CL-4B and Sephadex G-100 were supplied by Pharmacia Fine Chemicals. All other chemicals were reagent grade and used without further purification.

Protein determinations

Protein elution profiles from chromatographic procedures were estimated by monitoring absorbance at 280 nm. Protein content of all pooled fractions was quantified using the Hartree (1972) modification of the Lowry et al. (1951) procedure, with bovine serum albumin as the standard.

Inhibitor assay

α-Amylase and α-amylase inhibitor activities were measured by a modification of the method of Bernfeld (1955) as described by Frels and Rupnow (1984).

Purification of the inhibitors

Seeds of white kidney bean and red kidney bean were ground to a fine powder using a Janke and Kunkel Ika-Werk mill. Five grams of each sample were extracted by stirring for 2 hr in 125 mL 0.02M sodium phosphate buffer (pH 6.9), containing 0.15M NaCl. The extract was centrifuged at 14000 x g for 1 hr at 4°C. Insoluble matter was discarded and inhibitory activity was measured in the supernatant. Further purification included the following steps:

(1) **Ammonium sulfate fractionation.** The supernatant fluid collected after centrifugation was fractionated with ammonium sulfate. The protein fraction precipitated between 30 and 60% saturation was collected by centrifugation at 14000 x g for 1 hr at 4°C and resuspended in 0.02M sodium phosphate buffer (pH 6.9) at 25% saturation with ammonium sulfate.

(2) **Phenyl-Sepharose chromatography.** The protein solution obtained from the ammonium sulfate fractionation was applied to a 2.5 x 30 cm phenyl-Sepharose column which had previously been equilibrated with 0.02 M phosphate buffer (pH 6.9) at 25% saturation with ammonium sulfate. Unbound protein was eluted with elution buffer until absorbance at 280 nm approached zero. Bound protein was eluted with a 500 mL linear gradient of increasing ethylene glycol to 50% (v:v) and concurrently decreasing ammonium sulfate concentration to 0% saturation. Fractions containing more than 120 inhibitor units/mL were pooled and dialyzed against 0.02M sodium phosphate buffer (pH 6.9) containing 0.05M NaCl and then concentrated to approximately 4 mL by ultrafiltration using an Amicon PM-10 membrane.

(3) **Gel filtration on Sephadex G-100.** The concentrated inhibitor solutions were applied to a 2.0 x 120 cm column packed with Sephadex G-100 and equilibrated with 0.02M sodium phosphate buffer (pH 6.9).

Heat stability of inhibitors

The two purified α-amylase inhibitors from white kidney bean were tested for their stability to heat. Inhibitor-1 (I-1) and Inhibitor-2 (I-2) at activity levels of 5 units/mL and protein concentrations of 1.8×10^{-2} and 7.3×10^{-3} mg/mL, respectively, were heated at 70°C in 0.02M sodium phosphate buffer (pH 6.9) containing 0.05M NaCl. Aliquots of 0.525 mL were removed at various times after the 2 min come-up time, cooled in an ice bath for 10 min, and remaining inhibitory activity determined according to the usual procedure.
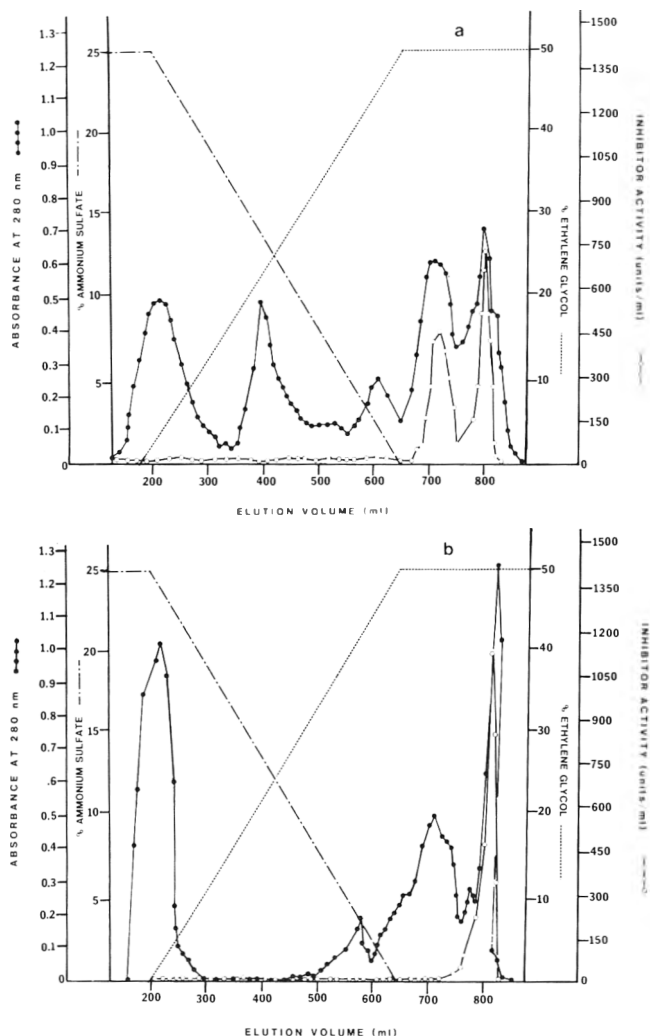
Fig. 1—Purification of α-amylase inhibitors from white (a) and red (b) kidney beans using phenyl-Sepharose chromatography.

Fig. 2—Effect of heat treatment at 70°C on purified α-amylase inhibitors I-1 (o) and I-2 (●) from white kidney bean.

RESULTS & DISCUSSION

White kidney bean

Phenyl-Sepharose chromatography resolved two distinct peaks with inhibitory activity which eluted when the concentration of ethylene glycol approached 50% (Fig. 1). These inhibitors were designated I-1 and I-2 according to their order of elution from the column. When both inhibitors were tested individually for their stability to heating at 70°C, I-1 proved to be less heat stable than I-2 (Fig. 2). After 20 min of heating, the inhibitory activity of I-1 was reduced by approximately 70%. I-2 retained 90% of its initial activity after 15 min and 20% after 150 min of heating. Marshall and Lauda (1975) and Pick and Wöber (1978) used heat treatments of 70°C for 15 min in the isolation of a single α-amylase inhibitor from white kidney bean. Results of this study would suggest that the identification of only one inhibitor and the 29% loss of inhibitor activity reported by Marshall and Lauda (1975) during the purifica-
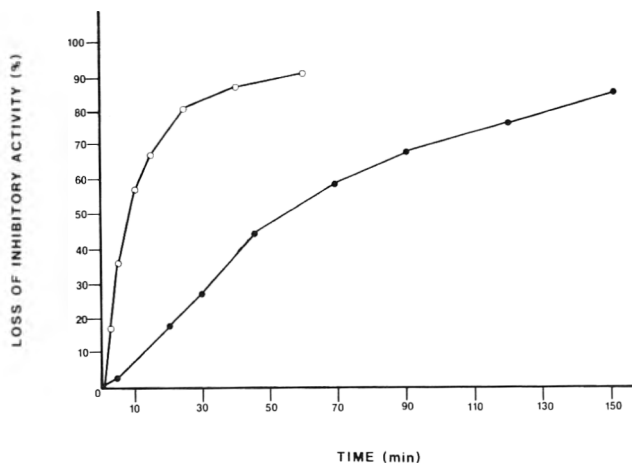
tion step was due to the destruction of a significant portion of I-1 during the heat treatment.

Red kidney bean

The α-amylase inhibitory activity isolated from red kidney bean eluted as a single peak from the phenyl-Sepharose column (Fig. 1). Further purification using gel filtration on Sephadex G-100 did not resolve a second inhibitor. Although Powers and Whitaker (1977) used a 60°C heat treatment for 20 min at pH 4.0 to purify the inhibitor, the results of this study indicate that a second, more heat labile α-amylase inhibitor is not present in red kidney bean.

REFERENCES

Bernfeld, P. 1955, Amylases, α and β. In "Methods in Enzymology," Vol. 1 (Ed.) S.P. Colowick and N.O. Kaplan. Academic Press, New York.
Frels, J.M. and Rupnow, J.H. 1984. Purification and partial characterization of of two α-amylase inhibitors from black bean (Phaseolus vulgaris). J. Food Biochem. 8: 281.
Granum, P.E. 1979. Studies on α-amylase inhibitors in foods. Food Chem. 4: 173.
Hartree, E.F. 1972. Determination of protein: a modification of the Lowry method that gives a linear photometric response. Anal. Biochem. 48: 422.
Jaffe, W.G., Moreno, R., and Wallis, V. 1973. Amylase inhibitors in legume seeds. Nutr. Rep. Inter. 7: 169.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265.
Marshall, J.J. 1975. α-Amylase inhibitors from plants. Am. Chem. Soc. Symposium Series 15: 244.
Marshall, J.J. and Lauda, C.M. 1975. Purification and properties of phaseolamin, an inhibitor of α-amylase, from the kidney bean, Phaseolus vulgaris. J. Biol. Chem. 250: 8030.
Pick, K.H. and Wöber, G. 1978. Proteinaceous α-amylase inhibitor from beans (Phaseolus vulgaris). Purification and characterization. Hoppe-Seyler's Z. Physiol. Chem. 359: 1371.
Powers, J.R. and Whitaker, J.R. 1977. Purification and some physical and chemical properties of red kidney bean (Phaseolus vulgaris) α-amylase inhibitor. J. Food Biochem. 1: 217.
Richardson, M. 1977. The proteinase inhibitors of plants and microorganisms. Phytochem. 16: 159.
Richardson, M. 1980-81. Protein inhibitors of enzymes. Food Chem. 6: 235.
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A Research Note

Effect of Magnesium Fertilizers on Total Glycoalkaloids and Nitrate-N in Katahdin Tubers

NELL I. MONDY and RATHY PONNAMPALAM

─────── ABSTRACT ───────

The effect of two sources of magnesium fertilization (56 kg/ha, MgO), epsom salt and dolomite, on total glycoalkaloid (TGA) and nitrate-nitrogen (NO_3-N) content of Katahdin potatoes was examined. Both magnesium fertilizers increased the TGA and NO_3-N content of tubers. Tubers grown in soils receiving epsom salt had significantly ($p < 0.05$) higher TGA content than the tubers receiving dolomite immediately following harvest, but after 5 mo storage, no significant differences were observed between the two Mg sources. The NO_3-N levels in tubers from plants receiving Mg fertilization were higher than the controls. Those receiving epsom salt were higher than those receiving dolomite. The NO_3-N levels decreased in all tubers after 5 mo storage. Cortex tissue was significantly ($p < 0.05$) higher in NO_3-N than the pith.

INTRODUCTION

POTATOES are more sensitive to magnesium deficiency than other crops (Bolton, 1977), yet such deficiencies may be corrected through the use of magnesium fertilizers such as magnesium sulfate (Houghland, 1964) or dolomite (Bolton, 1973). Conditions which lead to the development of Mg deficiency in plants are: acid, sandy, highly leached soils having low cation exchange capacity (CEC); calcareous soils, acid soils; and high rates of NH_4 or K fertilization (Kamprath and Foy, 1971). Magnesium is essential for plant growth and as a cofactor for several enzymes involved in photosynthesis, respiration, lipid and nitrogen metabolism (Gauch, 1972).

Fertilization practices affect the chemical composition of crops. $MgSO_4$ fertilization of potatoes increased total nitrogen, protein, and amino acids of tubers (Klein et al., 1982), as well as increased the yield and lipid content. Tubers from Mg-fertilized plant discolored less, were lower in phenols, and higher in lipids than the controls (Klein et al., 1981).

Although dolomite ($MgCO_3 \cdot CaCO_3$) is the primary source of Mg in many fertilizers (Brown, 1972), little information is available as to its effect on tuber quality. Since dolomite is slightly soluble and epsom salt ($MgSO_4 \cdot 7H_2O$) very soluble in water, the availability of Mg to the potato plant varies.

Glycoalkaloids, a class of naturally occurring toxicants present in potatoes, have been associated with bitter flavor (Sinden et al., 1976), inhibition of cholinesterase, and poisoning in humans and farm animals (McMillan and Thompson, 1979). Potatoes should contain no more than 20 mg glycoalkaloid per 100g fresh weight (Bomer and Mattis, 1924). Total glycoalkaloid (TGA) content of potatoes is influenced by cultivar, environment, fertilization practices, and mechanical and/or chemical-induced stress (Sinden et al., 1984). Fertilization with $MgSO_4$ increased TGA content of tubers (Evans and Mondy, 1984).

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High concentrations of nitrate in our food is of concern because of its precursor role in the formation of nitrites, which are involved in infant methemoglobinemia (Phillips, 1981), and which react with secondary or tertiary amines to form carcinogenic and mutagenic N-nitroso compounds (Walters et al., 1979). Potatoes contribute approximately 14% of the per capita ingestion of nitrates in the United States (White, 1975). Nitrates are influenced by cultivar, type and amount of nitrogen fertilizer, climate, moisture stress, and storage conditions (McDole and McMaster, 1978a, b; Augustin et al., 1977). Fong and Ulrich (1974) reported that $MgSO_4$ fertilization increased NO_3-N content of the potato plant, but no information was given for tubers.

This study was undertaken to determine the effect of different sources of Mg fertilization on TGA and the NO_3-N content of potato tubers.

MATERIALS & METHODS

KATAHDIN POTATOES grown at the Cornell Vegetable Research Farm in Riverhead, Long Island were used. Soil type was Haven fine sandy loam. Magnesium in the form of epsom salt ($MgSO_4 \cdot 7H_2O$) or dolomite ($MgCO_3 \cdot CaCO_3$) was banded at planting (56 kg/ha, magnesium oxide equivalent). Available mineral contents of these soils (kg/ha) averaged: magnesium 85.8, phosphorus 66.1, potassium 291.2, calcium 821.3, iron 14.1, and aluminum 180.7. Soil organic matter averaged 2.16% and soil pH was 5.73. All plots received the same amount of N-P-K fertilizer and were irrigated in the same manner.

Tubers were harvested 22 wk after planting and stored at 5°C for 5 mo after initial sampling. Four tubers were sampled for each analysis and duplicate analyses were made for each treatment. Tubers were sliced longitudinally from bud to stem end and divided into cortex and pith sections. Cortex is the area of greater metabolic activity. Both cortex and pith tissues were analyzed for NO_3-N content. Results were corrected for moisture content and reported on fresh weight basis.

Determination of toal glycoalkaloids

Analysis of fresh tubers was conducted using the modified titration method of Bushway et al. (1980).

Determination of NO_3-N

The NO_3-N content was determined by the phenoldisulfonic acid method using an aqueous extraction of fresh tubers (Ulrich et al., 1959).

Statistical analysis

Completely random design was employed and statistical significance of the data was determined using 3 x 2 x 2 or 3 x 2 analysis of variance with protected LSD test described by Steel and Torrie (1980).

RESULTS & DISCUSSION

WITH BOTH SOURCES of Mg fertilizer the TGA content of tubers immediately following harvest and after 5 mo storage was significantly ($p < 0.05$) higher than controls (Fig. 1). TGA was significantly ($p < 0.05$) higher in tubers from plants fertilized with epsom salt as compared to dolomite. This may have been due to greater availability of magnesium with epsom salt. Evans and Mondy (1984) sug-
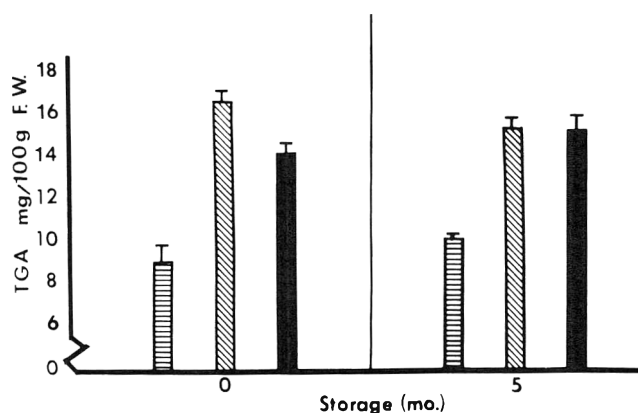
GLYCOALKALOIDS

Fig. 1—Effect of magnesium fertilization and storage on total glycoalkaloid content of Katahdin potato tubers: ⊟ Control; ⊠ Epsom; ■ Dolomite.

NITRATES

Fig. 2—Effect of magnesium fertilization and storage on NO_3-N content of Katahdin potato tubers. Cx - cortex; pi - pith. ⊟Control; ⊠Epsom; ■ Dolomite.

gested that Mg may increase glycoalkaloid synthesis by increasing chlorophyll production, stimulating sugar metabolism, and/or increasing amino acid synthesis.

Tubers from plants fertilized with either epsom salt or dolomite had higher ($p < 0.05$) NO_3-N than the control and cortex tissue was significantly ($p < 0.05$) higher in NO_3-N content than pith (Fig. 2). Klein et al. (1981) observed that fertilization with $MgSO_4$ increased total nitrogen, protein, and both the free and total amino acids of tubers.

After storage, tubers grown in plots receiving epsom salt or dolomite showed a significant ($p < 0.05$) decrease in NO_3-N content in the cortex tissue (Fig. 2). Augustin et al. (1977) reported that nitrate content of the tubers decreased slightly when stored for 5 – 7 months. With both sources of Mg fertilization potatoes stored for 5 mo increased in total nitorgen. Since nitrate is a precursor of protein synthesis and also nonprotein compounds, decreases in NO_3-N content following storage may have resulted from transformation of NO_3-N into other compounds. NO_3-N in tubers is apparently controlled by a complex mechanism since the interactions of treatment by tissue, treatment by storage, and tissue by storage were significant ($p < 0.05$).

Both epsom salt and dolomite increased the TGA and NO_3-N content of tubers when compared to controls, and the greater increase occurred with epsom salt. Selection of the type of magnesium fertilizer is important in determining tuber quality.

REFERENCES

Augustin, J., McDole, R.E., and Painter, G.C. 1977. Influence of fertilizer, irrigation, and storage treatments on nitrate-N content of potato tubers. Amer. Potato J. 54: 125.
Bolton, J. 1973. Sources of magnesium for sugar beet, potatoes, and wheat grown on an acid sandy soil at Woburn, beds. J. Agric. Sci. (Camb.) 81: 553.
Bolton, J. 1977. Liming effects on the response of potatoes and oats to phosphorus, potassium and magnesium fertilizers. J. Agric. Sci. (Camb.) 89: 87.
Bomer, A. and Mattis, H. 1924. The solanine content of potatoes. Zeitsch. Unters, Nahr. Genussa. Gebrauchsgegenstaende 47: 97.
Brown, L.D. 1972. Sources of magnesium as fertilizers. In "Magnesium in the Environment, Soils, Crops and Man," (Ed.) J.B.
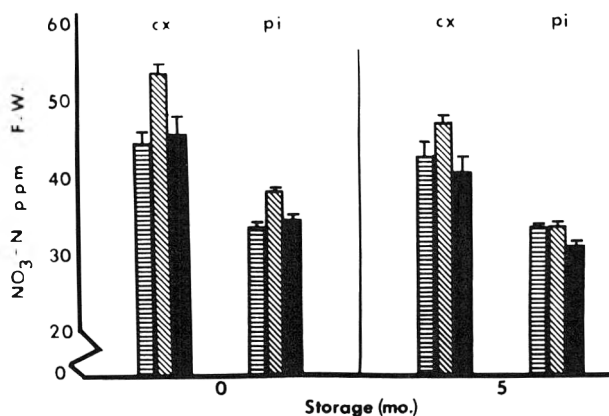Jones Jr., M.C. Blount, and S.R. Wilkinson, p. 251. Division of Agriculture, Fort Valley, GA.
Bushway, R.J., Wilson, A.M., and Bushway, A.A. 1980. Determination of total glycoalkaloids in potato tubers using a modified titration method. Amer. Potato J. 57: 561.
Evans, D. and Mondy, N.I. 1984. Effect of magnesium fertilization on glycoalkaloid formation in potato tubers. J. Agric. Food Chem. 32: 465.
Fong, K.H. and Ulrich, A. 1974. Magnesium nutrition of White Rose potato in relation to vegetable growth and minerals of leaf and root tissues. J. Amer. Soc. Hort. Sci. 99: 334.
Gauch, H.G. 1972. "Inorganic Plant Nutrition." Dowden, Hutchinson and Ross, Inc., Stroudsburg, PA.
Houghland, G.V.C. 1964. Hunger signs in crops. David McKay Company, NY.
Kamprath, E.J. and Foy, C.D. 1971. Lime-fertilizer-plant interactions in acid soils. In "Fertilizer Technology and Use," (Ed.) R.A. Olson, Soil Sci. Amer., Inc., p. 105.
Klein, L.B., Chandra, S., and Mondy, N.I. 1981. Effect of magnesium fertilization on the quality of potatoes. Yield, discoloration, phenols, and lipids. J. Agric. Food Chem. 29: 334.
Klein, L.B., Chandra, S., and Mondy, N.I. 1982. Effect of magnesium fertilization on the quality of potatoes: total nitrogen, nonprotein nitrogen, protein, amino acids, minerals, and firmness. J. Agric. Food Chem. 30: 754.
McDole, R.E. and McMaster, G.M. 1978a. Effects of moisture stress and nitrogen, protein, amino acids, minerals, and firmness. J. Agric. Food Chem. 30: 754.
McDole, R.E. and McMaster, G.M. 1978b. Effects of moisture stress and nitrogen fertilizatio on tuber nitrate-nitrogen content. Amer. Potato J. 56: 611.
McMillan, M. and Thompson, J.C. 1979. An outbreak of suspected solanine poisoning in school boys. Examination of criteria of solanine poisoning. Quart. J. Med. 48: 227.
Phillips, W.E.J. 1971. Naturally occurring nitrate and nitrite in foods in relation to infant methemoglobinemia. Food Cosmet. Toxicol. 9: 219.
Sinden, S.L., Deahl, K.L., and Aulenbach, B.B. 1976. Effect of glycoalkaloids and phenolics on potato flavor. J. Food Sci. 41: 520.
Sinden, S.L., Sanford, L.L., and Webb, R.E. 1984. Genetic and environmental control of potato glycoalkaloids. Amer. Potato J. 61: 141.
Steel, R.G.D. and Torrie, J.H. 1980. "Principles and Procedures of Statistics." McGraw-Hill Book Co., Inc., NY.
Ulrich, A., Rieri, D., Hills, F.J., George, A.G., Moore, M.D., and Johnson, C.M. 1959. Analytical methods for use in plants analysis. Calif. Agric. Expt. Stn. Bull. 766.
Walters, C.L., Carr, F.P.A., Dyke, C.S., Saxby, M.J., and Smith, P.L.R. 1979. Nitrite sources and nitrosamine formation in vitro and in vivo. Food Cosmet. Toxicol. 17: 473.
White, J.W. Jr. 1975. Relative significance of dietary sources of nitrate and nitrite. J. Agric. Food Chem. 23: 886.
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A Research Note

Proximate Composition of Sweet Potato Greens in Relation to Cultivar, Harvest Date, Crop Year and Processing

R. D. PACE, G. G. DULL, and B. R. PHILLS

————————ABSTRACT————————

Two sweet potato green cultivars, 'Jewel' and 'Carver', were produced during two crop years (1981 and 1982). The influence of cultivar, harvest date, crop year and processing on the proximate nutrient content of both varieties was determined. Results showed that overall nutrient content for both varieties were similar except for fat and ash content. Protein content in blanched and canned greens showed a decrease during the second and third collection periods, and the nutrient content from year to year remained constant. These data suggest that like other traditional vegetables, sweet potato greens can be preserved by traditional methods.

INTRODUCTION

THE CONSUMPTION of sweet potato greens as a fresh vegetable in many parts of the world (Thailand, Liberia, the Philippines, Indonesia, Malaysia) indicates that they are acceptable as food. Data have been reported (Villareal et al., 1978a, b, c) on the morphological, nutritional, and organoleptic properties of sweet potato greens for human consumption. In the United States, Ruiz et al. (1980) and Garlich et al. (1974) showed that the majority of the nutritional information on sweet potato greens is on its use in animal feed. Sweet potato greens are available during the hottest months of the year when traditional vegetable greens are unavailable and the effects of harvesting practices on nutrient content have not been widely studied. The objective was to study the effect of three independent variables, i.e. harvest date, crop year and processing on the nutrient quality (dependent variable) of two sweet potato cultivars.

MATERIALS & METHODS

EIGHT ROWS EACH of 'Carver' and 'jewel' sweet potato greens were randomly planted in an experimental plot and maintained under standard horticultural practices similar to those cited by Cuthbert and Jones (1978) with certain modifications. Field plots were subsoiled and cross disced to a depth of 25 cm. Twenty-seven kilograms of 10% mocap granules and 227.2 kg of 13:13:13 NPK were applied broadcast Preplant Incorporate with a tractor driven rototiller to a depth of 20.32 cm nine days prior to transplanting. Sweet potato sprouts 25.4 – 30.48 cm in length were planted on 20.32 cm raised beds 9.14m long × 1.21m wide. Sprouts were transplanted 10 – 13 cm deep by hand to insure accuracy of spacing and planting depth. Plots were cultivated twice prior to vining and all additional weeds were removed by hand hoeing.

During July-September, 1981 and 1982, sample collections were made three times (vegetative, mid-vegetative and tuberization phases) and each collection was separated by approximately four weeks. Ten centimeter tips of each of the two cultivars were collected on each harvest date and processed either by the blanching or canning procedures of Lopez (1969). In canning, 275g of fresh greens sample including stems and petioles were placed in a number 303

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can coated with bean enamel and 180g of 3% NaCl solution was added to each can.

An additional nonharvested treatment was added to the second crop year in an effort to compare the nutrient quality of multiple harvested greens to those harvested only at the end of the growing season.

Standard AOAC methods for plant materials, i.e. 3.117, 3.114, 3.115 and 3.004 (AOAC, 1980) for crude protein, crude fat, crude fiber, and ash, respectively, were used to determine the proximate composition of sweet potato greens. Ether-free extract was determined by difference. Nutrient content is reported on a dry weight basis.

The SAS System employing the Analysis of Variance and the Duncan's Multiple Range test at the 5% probability level was used to statistically analyze experimental differences among samples.

RESULTS & DISCUSSION

THE PROXIMATE COMPOSITIONS of the blanched sweet potato greens from two cultivars were quite similar (Table 1). Ash was significantly higher in 'Jewel' than in 'Carver' cultivars. This difference did not appear in the canned product (Table 2) probably because of the addition of salt. The fat level was significantly higher (5% probability) in canned 'Carver' than in 'Jewel' greens but the difference was not significant in the blanched product.

There were significant differences (5% probability) in protein and nitrogen free extract (NFE) values between harvest dates, protein being highest and NFE lowest in the first harvest. None of the other constituents was affected over the growing season. These results were the same for the blanched and canned products. The decrease in protein content measured throughout the growing season in these vegetables is the same as the data reported by Walter et al. (1978) and Villareal et al. (1978b).

Table 1—Proximate composition (% of dry wt) of blanched sweet potato greens from two cultivars harvested on three different dates and produced in two crop years

Cultivar	Year	Harvest period	Crude protein %	Crude fat %	Crude fiber %	Ash %	Nitrogen free extract %
'Carver'	1981	1	33.5	6.9	13.4	5.4	38.9
	1981	2	28.4	3.8	12.3	5.8	48.2
	1981	3	29.1	4.9	10.0	5.8	48.6
'Carver'	1982	1	34.5	4.0	10.9	5.6	44.2
	1982	2	26.1	5.1	11.4	5.2	51.4
	1982	3	28.3	4.3	10.1	5.9	50.7
'Jewel'	1981	1	35.2	6.1	10.8	6.4	39.6
	1981	2	28.8	4.1	12.6	5.8	47.1
	1981	3	28.2	3.6	12.0	6.4	47.4
'Jewel'	1982	1	32.1	5.2	13.1	6.3	41.7
	1982	2	26.2	3.0	12.5	7.2	50.4
	1982	3	28.8	4.2	11.6	7.0	47.3
		$\bar{X}$	29.9	4.6	11.7	6.1	45.9
		CV	4.1	22.1	9.4	8.0	3.8
		SE	1.2	1.0	1.1	0.5	1.7

Table 2—Proximate composition (% of dry wt) of canned sweet potato greens from two cultivars harvested on three different dates in the 1982 crop year

Cultivar	Harvest period	Crude protein %	Crude fat %	Crude fiber %	Ash %	Nigrogen free free %
'Carver'	1	28.2	7.4	10.0	20.8	30.2
	2	21.8	7.1	13.3	22.6	32.2
	3	23.2	7.5	11.3	20.6	34.6
'Jewel'	1	25.9	6.2	10.8	23.5	30.6
	2	20.8	6.4	9.8	21.7	38.4
	3	24.3	6.4	10.2	21.3	34.9
	X̄	23.9	6.7	10.7	21.6	34.0
	CV	7.0	14.1	22.7	6.0	8.7
	SE	1.7	1.0	2.4	1.3	2.9

It might be expected that if major differences in nutrients occurred, they would be between crop years owing to variable environmental conditions. Due to field production problems it was impossible to obtain sufficient plant material for the canned products in 1981; therefore, comparisons between the years could not be made. The only significant difference (5% probability) between crop years for the blanched product was for NFE, which was lower in 1981 than in 1982. This difference was true for both cultivars.

In the treatment added to compare multiple harvests with nonharvested plants during the season it was found that the proximate composition of both cultivars was essentially the same (data not shown). This is a positive factor in favor of multiple harvests of greens from the same plants.

Canned sweet potato greens have a proximate composition which is similar to that of canned spinach and turnip greens. The canned sweet potato greens contained on a percent dry weight basis 23.9% crude protein, 6.7% crude fat, 10.7% crude fiber, 21.6% ash and 34.0% nitrogen free extract. These nutrient values were similar when compared to the respective values for spinach and turnips as taken from USDA Handbook #8: crude protein — 28.6% and 22.8%; crude fat — 5.7% and 4.8%; crude fiber — 10.0% and 9.5%; ash — 22.9% and 20.6%; and nitrogen-free extract — 33.0% and 40.0%. Interestingly, in our laboratories

at Tuskegee Institute, it was found that sweet potato greens are similar in taste to spinach.

CONCLUSION

NUTRIENT CONTENT in greens from two sweet potato cultivars was not greatly affected by processing; harvested vegetables during periods 2 and 3 which were processed tended to have decreased protein content in 10 cm tips; and the nutrient content is likely to remain the same from year to year. This information as collected provides valuable data for consumers, processors and producers. The fact that sweet potato greens are consumed in some countries and that the vegetable lends itself readily to traditional preservation methods indicates that they have potential as a new food in the U.S.

REFERENCES

AOAC. 1980. "Official Methods of Analysis," 23rd ed. Association of Official Analytical Chemists, Washington, DC.
Cuthbert, F.P. and Jones, A. 1978. Insect resistance as an adjunct or alternative to insecticides for control of sweet potato soil insects. J. Amer. Soc. Hort. Sci. 103: 443.
Garlich, J.D., Bryant, D.M., Covington, H.M., Chamblee, D.S., and Purcell, A.E. 1974. Skin pigmentations with sweet potato vine meal. Poultry Sci. 53: 692.
Lopez, A. 1969. "A Complete Course in Canning." 9th ed. The Canning Trade, Baltimore, MD.
Ruiz, M.E., Pezo, D., and Martinez, L. 1980. The use of sweet potato (Ipomoea batatas, (L) (Lam) in animal feeding 1. Agronomic aspects. Tropical Animal production 5: 144.
Villareal R.L., Lin, S.K., Chang, L.S., and Lai, S.H. 1978a. Use of sweet potato (Ipomoea batatas) leaf tips as vegetables 1. Evaluation of morphological traits. Experimental Agric. 15: 113.
Villareal, R.L., Tsou, S.C.S., Lin, S.K., and Chiu, S.C. 1978b. Use of sweet potato (Ipomoea batatas) leaf tips as vegetable. 2. Evaluation of yield and nutritive quality. Experimental Agric. 15: 117.
Villareal, R.L., Tsou, S.C., Chiu, S.C., and Lai, S.H. 1978c. Use of sweet potato (Ipomoea batatas) leaf tips as vegetables. 3. Organoleptic evaluation. Experimental Agric. 15: 123.
Walter, W.M., Purcell, A.E., and McCollum, G.K. 1978. Laboratory preparation of a protein-xanthophyll concentrate from sweet potato leaves. J. Agric. Food Chem. 26: 1222.
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A Research Note
A Puncture Testing Method for Monitoring Solid Substrate Fermentation

H. J. KRONENBERG and Y. D. HANG

─────────── ABSTRACT ───────────

Meitauza, an indigenous Chinese food made from fermented okara (soymilk residue), was used as a model system for monitoring solid substrate fermentations. The Instron Universal Testing machine and Chatillon hand operated force gauge were used to measure the force to penetrate meitauza as an estimation of mycelial binding. Increased firmness during fermentation was caused by fungal growth and not by water loss and surface crusting. Puncture force readings with the Instron were werll correlated (r = 0.86) with Chatillon measurements. The results of this study indicate that textural measurements using puncture force may have valuable application in monitoring the degree of mycelial growth or fermentation in a solid substrate.

INTRODUCTION

SOLID SUBSTRATE FERMENTATION refers to any fermentation that occurs on a solid or semi-solid substrate and has been used in the production and preservation of a variety of indigenous fermented foods such as cheese, meitauza, soy sauce, sufu and tempeh (Aido et al., 1982; Kronenberg and Hang, 1984). A major difficulty associated with solid substrate fermentation is lack of direct analytical methods. Current methods of investigaton include enzyme assays, monitoring CO_2/O_2 balance, estimation of fungal chitin and infrared reflectance techniques (Aido et al., 1982; Narahara et al., 1982; Rathbun and Shuler, 1983).

Fungal mycelium has a cohesive structure which contributes a meat-like texture (Worgan, 1976). The process of substrate invasion and binding by the fungal mycelium is crucial to the production of meat-like textures important to indigenous fermented foods (Steinkraus, 1982). Puncture testing is an empirical method used in textural studies of food (Bourne, 1982). It measures the force required to push a metal probe into food to a depth that causes irreversible crushing or flow of the sample. The purpose of this study was to develop an objective technique for monitoring mycelial growth or fermentation in a solid substrate. Meitauza, an indigenous fermented food prepared by culturing *Actinomucor elegans* on okara (Hesseltine, 1965; Kronenberg and Hang, 1984), was used as a model system.

MATERIALS & METHODS

Meitauza fermentation

The method of producing meitauza was described by Kronenberg and Hang (1984). Okara was inoculated with an appropriate amount of inoculum (5.5 x 10^6 viable spores/kg) and formed into cakes using petri dishes as molds (13 x 53 mm diameter). The cakes were fermented at 15°C on trays in an incubator and the humidity was maintained at 75% during the meitauza fermentation.

Instron puncture test

Measurements of the sample texture were made with an Instron Universal Testing machine equipped with a 0.17 cm diameter punch attached to the crosshead and driven vertically at 20 cm/min

(Bourne, 1982). The force recording system was calibrated at 1 or 2 Newtons depending on the sample texture. Samples were punched while laying flat on a metal plate and the maximum puncture force (Newtons) measured from the force-distance curves. Determinations were made at 0, 30, 50, 70, and 80 hr fermentation, and each cake measured four times by punching at the corners (positions 1 to 4) of a centered cardboard template (Fig. 1).

Chatillon puncture test

The protocol for measurement of firmness with a hand-operated puncture tester followed guidelines listed by Bourne (1982). A Chatillon force gauge (Model No. 516-500, John Chatillon & Sons, Inc., Kew Gardens, NY), calibrated from 0 – 500g in 5g increments, was used for hand measurements. A 0.18 cm diameter flat-ended punch was mounted in the chuck of the gauge.

A comparison of Instron and Chatillon methods was accomplished by first punching each cake in positions A and B on the cardboard template (Fig. 1) by hand. Cakes were then immediately punched with the Instron Universal machine at positions 1 through 4. Measurements made with both instruments were each averaged for each cake, and results pooled from two fermentation trials to give 30 paired observations for comparing the readings.

Statistical analysis

Experimental design was organized as follows: blocking according to fermentation trials; nine treatments representing 0, 30, 50, 70, and 80 hr incubation times for okara and 30, 50, 70, and 80 hr incubation times for meitauza; and two replicates per treatment, each consisting of 12 measurements of three cakes (subsamples) from one batch. Two-way factorial analysis of variance was constructed with fermentation (two levels, inoculated versus uninoculated samples) and incubation time (four levels, 30, 50, 70, 80 hr)
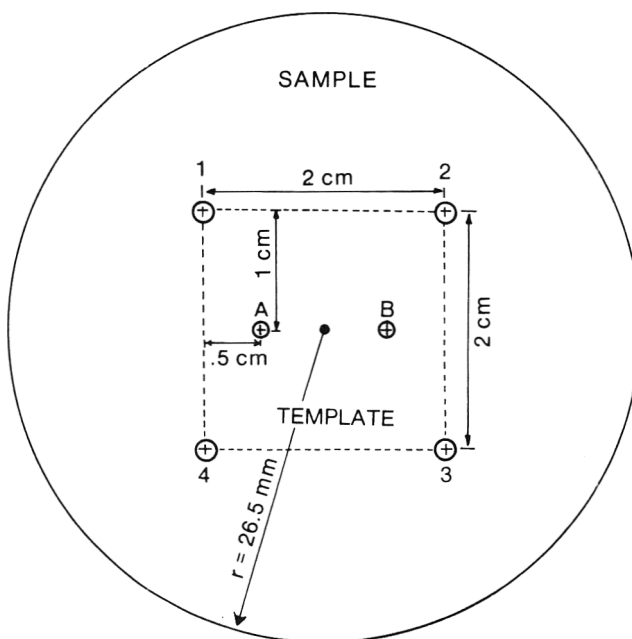

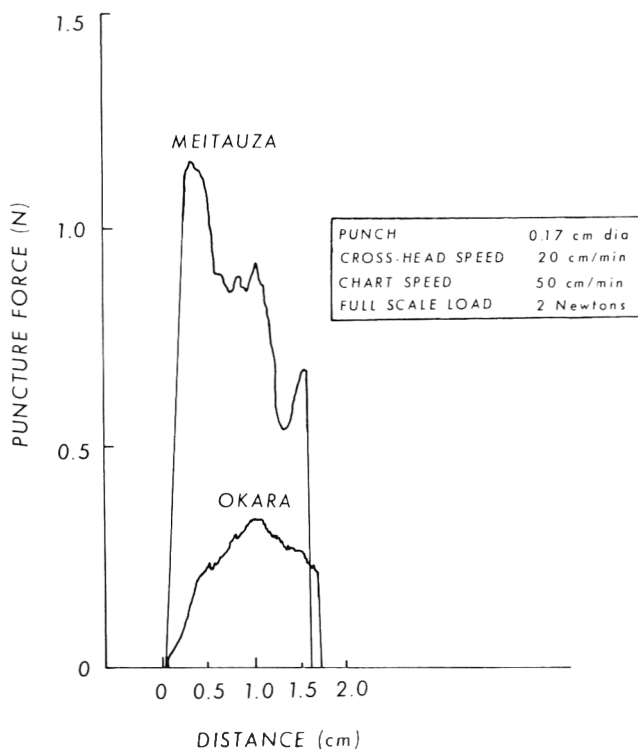Fig. 1—Template for puncture testing. Positions A, B: Chatillon. Positions 1, 2, 3, 4: Instron.

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Fig. 2—Instron force-distance curves for okara and meitauza.

RESULTS & DISCUSSION

DURING FERMENTATION, the firmness of meitauza measured with the Instron Universal Testing machine increased with fermentation time. At 30 hr, the puncture force of meitauza commenced to increase rapidly, and by 70 hr, there was a pronounced difference in the puncture force of meitauza and okara. Fig. 2 shows a comparison of force-distance curves generated with the Instron instrument for okara and meitauza. Friable cakes of okara produced curves during puncture tests characteristic of a soft yet relatively homogenous material. The maximum force of the single peak probably indicates how tightly the residue was packed in petri dish molds. In contrast, the force-distance curve for meitauza had a major peak followed by several maxima and minima. The maximum force was interpreted to represent overall firmness, while subsequent smaller peaks are attributed to the nonuniform process of mycelial growth and perhaps large fragments of soybean cotyledons

in the okara. The puncture testing results approximately paralleled the rise in pH, marked increase in nonprotein nitrogen and production of a large amount of acid protease (Kronenberg and Hang, 1984). The optimum fermentation time for neitauza was 70 hr. Beyond 70 hr the sensory properties of meitauza deteriorated rapidly because of the formation of an appreciable amount of ammonia.

Analysis of the variance of the interaction ($P \leqslant 0.05$) of fermentation x incuabion time indicates that the increase in puncture force of meitauza was due to mycelial binding and can be distinguished from increasing firmness of okara during incubation which was probably caused by water loss and surface crusting. Analysis of the linear component of fermentation x incubation time shows that the rate of increase of firmness in meitauza was significantly greater ($P \leqslant 0.01$) than that of okara.

Measurements take by the Instron Universal Testing machine and the Chatillon gauge were highly correlated ($r = 0.86$), with 95% confidence limits calculated as $0.724 \leqslant p \leqslant 0.932$. Thus, the Chatillon gauge can be used as a simpler method for measuring puncture force during the meitauza fermentation. The results of this study indicate that textural analysis with puncture force as the measured parameter may be a valuable technique in monitoring the degree of mycelial growth or fermentation in a solid substrate.

REFERENCES

Aido, K.E., Hendry, R. and Wood, B.J.B. 1982. Solid substrate fermentations. Adv. Appl. Microbiol. 28: 201.
Bourne, M.C. 1982. "Food Texture and Viscosity: Concept and Measurement." Academic Press, New York.
Hesseltine, C.W. 1965. A millennium of fungi, food, and fermentation. Mycologia 57: 149.
Kronenberg, H.J. and Hang, Y.D. 1984. Biochemical changes in okara during meitauza fermentation. Nutrition Reports International 30: 439.
Narahara, H., Koyama, Y., Yoshida, T., Pichangkura, S., Veda, R., and Taguchi, H. 1982. Growth and enzyme production in solid-state culture of Aspergillus oryzae. J. Ferment. Technol. 60: 311.
Rathbun, B.L. and Shuler, M.L. 1983. Heat and mass transfer effects in static solid-substrate fermentations: design of fermentation chambers. Biotechnol. Bioeng. 25: 929.
Steinkraus, K.H. 1982. The indigenous fermented foods. In Nestle Research News 1980/81, (Ed.) C. Boella. Nestle Co. Ltd., La Tour-de-Peilz, Switzerland.
Worgan, J.T. 1976. Wastes from crop plants as raw material, for conversion by fungi to food or feedstock. In "Food From Wastes," (Ed.) G.G. Birch, K.J. Parker, and J.T. Worgan. Applied Science Publisher, London.
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A Research Note

Determination of Phytic Acid in Foods by Ion Chromatography with Post-Column Derivatization

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————————ABSTRACT————————

An ion chromatographic method to determine phytic acid in foods, which allows for the direct injection of extracts into the column without need of a prepurification step, was developed. Infant formula powder, soy flour, soy isolate, wheat bran, and wheat bread were analyzed using the new ion chromatographic method and an ion exchange method. Phytic acid determined with the ion chromatographic method ranged from 0.2% for infant formula powder to 3.28% for wheat bran. The generally lower values found using the ion chromatographic method compared to the ion exchange method are attributed to the measurement of interfering substances such as breakdown products of phytic acid by the ion exchange procedure.

INTRODUCTION

PHYTIC ACID (myo-inositol hexaphosphate) is currently receiving considerable attention because of its effect on the bioavailability of certain minerals as well as the need for methods of determination. The nutritional data are often confusing. Phytic acid has been observed to be detrimental to the absorption of minerals such as zinc (Oberleas, 1983). Recently, it was found to enhance the absorption of iron in rats (Gordon and Chao, 1984). Lyon (1984) points out that components in the diet such as other complexing ligands probably help control mineral bioavailability and might account for the conflicting results in the literature. While phytic acid is generally viewed in the United States as an antinutrient, in other countries it is used extensively as a food additive for antioxidant and other applications (Graf, 1983).

A prerequisite for studying the nutritional effects of phytic acid is to have an accurate method to measure it. Before 1980 phytic acid was assayed exclusively by nonspecific precipitation or ion exchange procedures (Thompson and Erdman, 1982). Since then several reversed-phase high performance liquid chromatographic (HPLC) procedures have been published, the most recent using ion-pair chromatography (Lee and Abendroth, 1983). Problems encountered with phytic acid eluting in the solvent front were solved using ion-pairing, but the system still required an ion exchange prepurification step before injection. The objective of this paper was to establish a direct injection method for phytic acid determination in which the ion exchange mode previously used for prepurification is used for the analytical separation.

MATERIALS & METHODS

Materials

Dodecasodium phytate was obtained from Sigma Chemical Co. (St. Louis, MO). Standard sodium phytate (Sigma Chemical Co.) was measured to contain 102% by ion chromatography and 95% by ion exchange of the amount stated on the label. Infant formula powder and wheat bread were commercial brands. Soy flour and soy isolate were obtained from A.E. Staley Manufacturing Co.

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(Decatur, IL) and Ralston Purina Co. (St. Louis, MO), respectively. Wheat bran was AACC certified.

Apparatus

Ion separator AG3 (guard) and AS3 (analytical) columns (Dionex Corp., Sunnyvale, CA) were installed in a Dionex Model 12 Auto Ion Analyzer. The eluant, 0.11M HNO_3, was continuously purged with a slow stream of helium to prevent air bubble formation. A 2-μm inlet filter was attached to the forward end of the eluant line. Eluant was pumped at a rate of 1 mL/min. The volume of the injection loop was 100 μL. The effluent from the analytical column was directed into a Dionex post-column reactor, where it was combined with 0.1% $Fe(NO_3)_3$ in 2% $HClO_4$. A total flow rate of 1.5 mL/min was maintained. The mixture entered a Model 8-200 Sargent-Welch Pye Unicam UV/visible spectrophotometer equipped with a flow cell; absorbance was monitored at 290 nm.

Sample preparation

Bread was dried in an 85°C oven and homogenized in a Waring Blendor; the other foods were extracted as received. Five grams of sample was placed in a 200-mL Nalgene bottle and 100 mL of 1.2% HCl was added. The mixtures were shaken 30 min in a mechanical shaker and then centrifuged at 25,000 rpm in the 28 rotor of a Beckman L2-65B ultracentrifuge for 30 min. The fat layer was discarded and the remaining supernatant was collected and filtered through a 0.45-μm Millipore filter. For ion chromatography, aliquots were diluted to contain between 5 and 25 μg of phytic acid/ 100 μL.

Comparative analyses

Foods prepared as described above were analyzed by ion chromatography and by the ion exchange procedure of Ellis and Morris (1983). In the latter, phosphate was measured by Bartlett's (1959) method.

RESULTS & DISCUSSION

THE ION CHROMATOGRAPHIC METHOD was inspired by Fitchett and Woodruff's (1983) procedure for measuring polyphosphates in detergents using ferric iron as a derivatizing reagent (Imanari et al., 1982). Although Fitchett and Woodruff (1983) initially used 330 nm for detection, we discovered that the ferric phytate complex has an absorption maximum at 290 nm. While the post-column reaction is not specific for phosphate compounds, we observed that, in the foods analyzed, phytate eluted cleanly as the last peak.

The retention time of phytic acid is determined by the concentration of the HNO_3 eluant. In this system, with 0.11M HNO_3 as the eluant, phytic acid elutes in 7 min with an interval of 10 min between injections (Fig. 1). Resolution of the phytate peak can be improved by decreasing the eluant strength, but this increases the retention time and decreases the sensitivity.

As commonly occurs in HPLC, the peak heights of standards often did not remain constant throughout the day. To compensate for this, analyses were conducted in morning or afternoon batches. In any batch, each test solution and a standard solution were assayed in duplicate in an order such that duplicates were assayed equal distances from the middle of the batch. For example, if 12 injections were to be made, numbers four and nine would be standards.
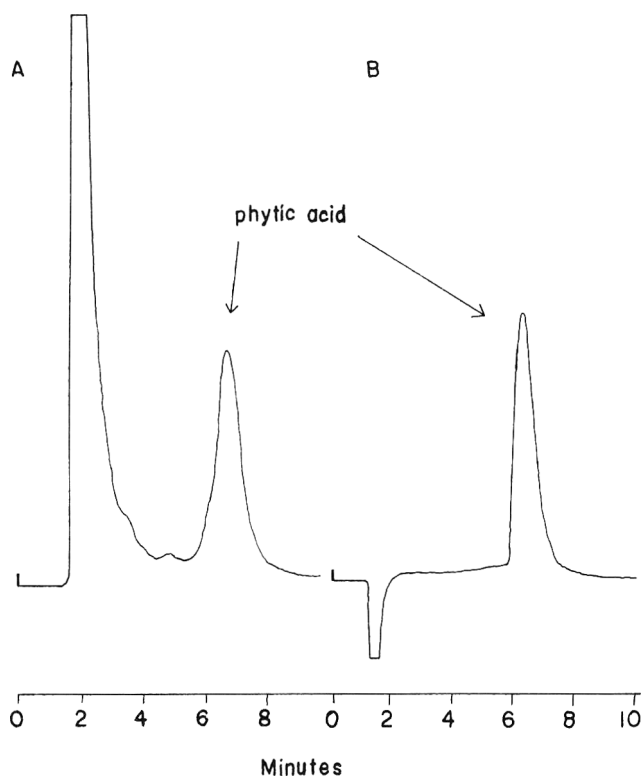
Fig. 1—Phytic acid determination by ion chromatography: (A) infant formula; (B) 25 μg of phytic acid.

Recovery studies were performed with a powdered infant formula that contained 0.225% phytic acid as measured by ion chromatography. Standards were added to the powder in amounts to give additions of 0.050, 0.100, 0.250, 0.500, and 1.000%. Recoveries were 96 ± 4%.

Phytic acid was measured in several foods by both ion chromatography and the Ellis and Morris (1983) ion exchange procedure. The results in Table 1 show fairly good agreement. Some values obtained by ion chromatography were somewhat lower than by the Ellis and Morris procedure. A reasonable explanation is that ion chromatography specifically measures phytic acid, whereas the Ellis and Morris procedure is not specific and may detect additional components of the foods. This is likely considering that inorganic phosphate is eluted with 0.1M NaCl before the phytate is recovered with 0.7M NaCl. Everything eluting between 0.1 and 0.7M NaCl would be in the phytate

Table 1—Phytic acid content of foods determined by ion chromatography and by the Ellis and Morris ion exchange method

Food	Phytic acid, %[a]	
	Ion chromatography	Ion exchange
Infant formula powder	0.20 ± 0.02[b]	0.31 ± 0.02
Soy flour	1.66 ± 0.07	1.75 ± 0.05
Soy isolate	1.38 ± 0.04	1.60 ± 0.02
Wheat bran	3.28 ± 0.14	3.23 ± 0.11
Wheat bread	0.74 ± 0.04	0.79 ± 0.04

[a] Duplicate extracts of each food were prepared; each extract was analyzed in duplicate by each procedure.
[b] ± Standard deviation.

fraction. Interfering substances could include hydrolysis products of phytic acid such as inositol pentaphosphate, inositol tetraphosphate, etc., which have been found to be present in bread (Nayini and Markakis, 1983a, b). Studies in our laboratory have indicated that small amounts of inositol pentaphosphate are present in soy-based infant formulas. The lower inositol phosphates have been shown to precipitate with iron (de Boland et al., 1975) and thus could also interfere with the precipitation methods of phytate determination.

REFERENCES

Bartlett, G.R. 1959. Phosphorus assay in column chromatography. J. Biol. Chem. 234: 466.
de Boland, A.R., Garner, G.B., and O'Dell, B.L. 1975. Identification and properties of "phytate" in cereal grains and oilseed products. J. Agric. Food Chem. 23: 1186.
Ellis, R. and Morris, E.R. 1983. Improved ion-exchange phytate method. Cereal Chem. 60: 121.
Fitchett, A.W. and Woodruff, A. 1983. Determination of polyvalent anions by ion chromatography. Liq. Chromatogr. HPLC Mag. p. 1: 48.
Gordon, D.T. and Chao, L.S. 1984. Relationship of components in wheat bran and spinach to iron bioavailability in the anemic rat. J. Nutr. 114: 526.
Graf, E. 1983. Applications of phytic acid. J. Am. Oil Chem. Soc. 60: 1861.
Imanari, T., Tanabe, S., Toida, T., and Kawanishi, T. 1982. High-performance liquid chromatography of inorganic anions using Fe^{3+} as a detection reagent. J. Chromatogr. 250: 55.
Lee, K. and Abendroth, J.A. 1983. High performance liquid chromatographic determination of phytic acid in foods. J. Food Sci. 48: 1344.
Lyon, D.B. 1984. Studies on the solubility of Ca, Mg, Zn, and Cu in cereal products. Am. J. Clin. Nutr. 39: 190.
Nayini, N.R. and Markakis, P. 1983a. Effect of fermentation time on the inositol phosphates of bread. J. Food Sci. 48: 262.
Nayini, N.R. and Markakis, P. 1983b. Effect of milling extraction on the inositol phosphates of wheat flour and bread. J. Food Sci. 48: 1384.
Oberleas, D. 1983. The role of phytate in zinc bioavailability and homeostasis. In "Nutritional Bioavailability of Zinc," (Ed.) G.E. Inglett, p. 145. American Chemical Society, Washington, DC.
Thompson, D.B. and Erdman, J.W. Jr. 1982. Phytic acid determination in soybeans. J. Food Sci. 47: 513.
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A Research Note
Computerized Procedure for Time-Intensity Sensory Measurements

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—————————————ABSTRACT—————————————

A computerized procedure for time-intensity (TI) measurements was developed wherein instructions to the judge as well as data collection were handled by a microcomputer. A joystick linked to the computer was used by the judge as an input device for recording perceived intensity of taste with time. TI curves for bitterness of iso-α-acids in water using both the computerized system and a strip chart recorder showed no significant differences in maximum intensity, time to reach maximum intensity, and duration of bitterness. The computerized system, however, was faster, more accurate, and more interesting for the judges.

INTRODUCTION

COMPUTERS have been used for analysis of sensory data (Aust, 1984; Brady, 1984; Savoca, 1984), but few published methods involve computers in the data-collection phase. Duffield and Stagg (1983) had judges score directly on cards, using a "mark sense" card reader connected to a microcomputer. McLellan and Cash (1983) installed a microcomputer in a sensory booth and had judges place marks on an unsegmented line, displayed on the terminal, by moving an arrow. The computer recorded the "intensity" under the coding for the sensory characteristic, judge, and sample number. Pecore (1984) had consumers enter responses via a joystick linked to a microcomputer with floppy disks for questionnaires and data storage. This reduces session duration, increases accuracy of data collection, gives consistent averaging, allows automatic comparison with previous results and flexibility due to data stored on disks.

Early time-intensity (TI) protocols had judges trace intensity with time on graph paper (Nielson, 1957) or record numerical intensities on score sheets at 1-sec intervals (Jellinek, 1964). Later, a scale was placed over the moving paper of a chart recorder which monitored time (Larson-Powers and Pangborn, 1978). McLellan (1984) recommended use of microcomputers for time-dependent measurements but there has been little application to TI. Schmitt et al. (1984) used a digitizer as an input device for transferring judge's TI curves to a computer, which provided more data points and more precise statistical analyses.

The purpose of the present investigation was to test a system wherein instructions and data collection were handled by a microcomputer. The technique was contrasted with data collected by chart recorders, using bitterness of iso-α-acids in water.

METHODS & MATERIALS

Instrumentation

Judges used a joystick interfaced to a microcomputer to record taste intensity of stimuli as a function of time in the mouth. The joystick functioned as a variable resistor which produced an analog voltage directly proportional to the position of the stick in the slot, which was labelled "none" and "extreme" at the ends (Fig. 1).

A button on the joystick box was used to signal the microcomputer at the onset and end of a test. A computer terminal displayed instructions to the judge at the beginning of a session as well as at the end of fixed time periods, e.g., expectoration time and time between replicates.

The microcomputer data system, based on a 16 bit LSI 11/2 microprocessor (Digital Equipment Corporation [DEC], Maynard, MA), contained a parallel interface (Model DRV11, DEC), an analog to digital converter (ADC) (Model 1030, ADAC Corporation, Woburn, MA), and two memory modules (Model MXV11-AC, DEC). The parallel interface connected the joystick push button to the microcomputer. The ADC digitized the position of the joystock (assigned a maximum of 100 = "extreme") and transferred the reading to the microcomputer. The memory modules provided 56K bytes random access memory, a start-up program, four serial ports, and a 60-cycle crystal clock for accurate timing of the program modules. A dual cartridge tape drive (Model TU58-KDEC) was used as a low-cost, mass memory storage device and provided random access to block-formatted data on pocket-size tape cartridges for program and data storage. After collection and storage, the data were transferred electronically to a large microcomputer based on the LSI 11/23 microprocessor. This microcomputer, a multi-user system with the RSX-11M operating system (DEC), had large amounts of hard disk storage space.

Computer programs

Programs controlling data acquisition were written in FORTRAN IV and assembly languages. They were developed, tested, and used with the RT-11, version 4.0, operating system (DEC). The program modules which handled the exchange of information by the terminal and stored collected data on tape cartridges also were written in FORTRAN. The program module which acquired the TI input from the joystick was written in assembly code. Some data reduction was performed on the RSX-11M operating system via Minitab (University Park, PA), and graphic outputs were obtained via Multiware Graphics (Multiware Inc., Davis, CA).
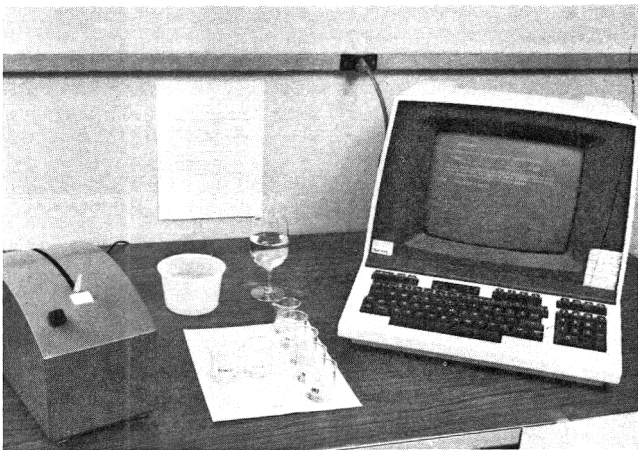

Fig. 1—Computerized sensory evaluation station showing the joystick for the judge to enter the TI response, the experimental samples, and the computer terminal which displays instructions to the judge.
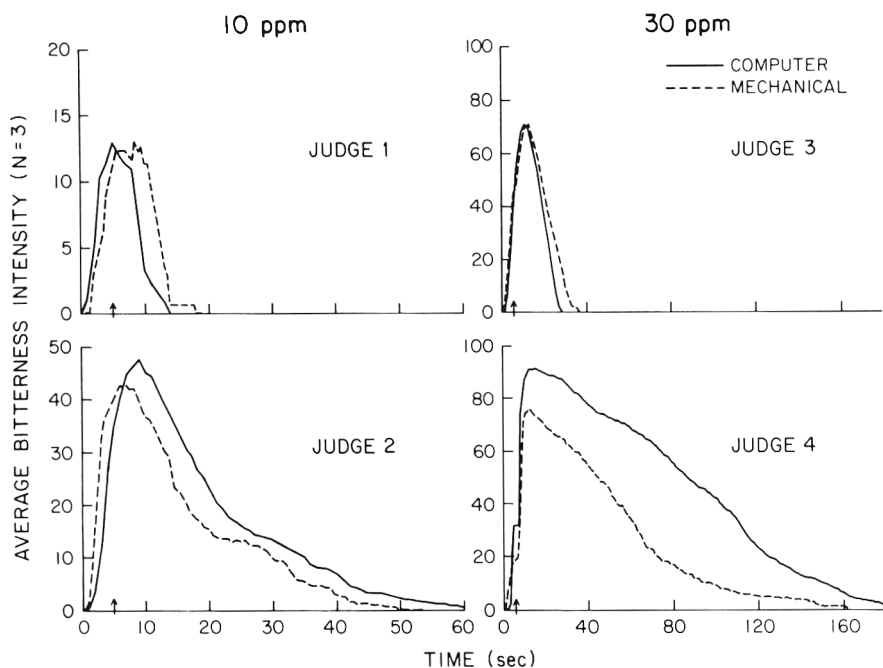
Fig. 2—TI curves for average bitterness intensity responses to 10 and 30 ppm of iso-α-acids in water by four representative judges. Results from computer and mechanical methods are indicated by solid and broken lines, respectively. The arrows on the time axes represent expectoration of the samples.

Judge Instruction

The terminal and joystick box were placed on a table with the microcomputer positioned underneath, out of sight of the judge (Fig. 1). A coded tray of samples was placed on the table just before the judge arrived, along with rinse water and a cuspidor. First the judge typed the title of the program to be run on the terminal keyboard. A 90-sec interval elapsed before the instructions appeared on the screen during which the judge tasted two reference samples corresponding to "none" and "extreme" intensities. The instructions asked the judge to calibrate the internal computer scale by placing the joystick at one end of the scale, pressing the button, then placing the joystick at the other end and re-pressing the button. The screen instructed the judge to enter the number of samples to be tested, reminded the judge of the main instructions and started a 30-sec countdown displaying the remaining time. Then, the judge placed the first sample in his mouth, pressed the button, and started recording perceived intensity by moving the joystick along the scale. An auditory signal ("beep") at a pre-programed time, e.g., 5 sec., signaled the judge to swallow (or expectorate) the sample, while continuing to move the joystick to represent perceived intensity. When the joystick was brought back to "none", the judge re-pressed the button, starting the countdown for the next sample. At the end of the session, the judge entered his initials and the three-digit code of the sample tray. The resultant data were saved on a cartridge tape.

To check responses, the digitized entries (at 1/2-sec intervals) could be displayed on the screen. After each day's runs, datafiles were transferred to the hard disk on the larger microcomputer. At any time, data could be displayed as digits on the terminal and/or runoff on a printer, or transferred to a graphics printer for display and/or runoff. The datafiles also were readable by user-written programs or by prewritten application packages, e.g., MINITAB.

Application to measurement of bitterness

Using a balanced, complete-block design, 10, 20, and 30 ppm of iso-α-acids ('Haas Isomerized Hop Extract' with 30% iso-α-acids, supplied by Dr. F.L. Rigby, John I. Haas, Inc., Yakima, WA.) in distilled water were evaluated in triplicate. Samples of 15 mL were served in coded, 50-mL beakers in random order. Distilled water was used for oral rinsing between samples. Tests were conducted in an isolated room maintained at 21 ± 2°C, under fluorescent illumination. At separate times, one session was done with the computer and one with the mechanical recorder (Heathkit, Benton Harbor, MI) where time was monitored by a modified strip chart recorder (Larson-Powers and Pangborn, 1978). Perceived bitterness intensity was recorded manually with a pen on a 100-division scale labelled "none" to "extreme" on the stationary paper cutter strip.

Eleven judges participated, selected on the basis of interest and availability. After a training session, half of the judges started with

the computerized system, the other half with the mechanical one.

RESULTS

FIG. 2 shows representative TI bitterness curves for four judges in response to two concentrations of iso-α-acids in water obtained by the two systems. Although individual judges gave different maxima and different total duration of bitterness, similar tracings were obtained within a judge by computer and mechanical systems. Across the eleven judges tested, there were no significant differences between computer and mechanical data for maximum intensity, time to maximum intensity, or total duration (Student's paired t-test, 10 df).

CONCLUSIONS

THE COMPUTERIZED and mechanical TI procedures gave similar, and in some cases, identical results, but the former offered many advantages: the joystick afforded the experimenter better control of the test conditions, i.e., time between successive samples and between sample intake and expectoration or swallowing. Also, there was a substantial reduction in labor and potential human errors associated with hand-conversion of TI curves into numbers for statistical analysis. The joystick facilitated expression of perceived intensity by providing a highly personalized communication between the judge and the computer and the judge was not distracted by a moving chart or biased by seeing his previous responses. Whereas the mechanical system allows judges to draw curves whose shapes they might reproduce by habit, the computerized system precludes conceptualizing the shape of the curve being generated. Also, computerization permits automatic printout of the data as digits or as TI curves, the data files may be submitted directly to statistical or other basic mathematical analyses, and little data storage space is required. Judges commented that the computerized system is fun. This increased their motivation and interest for daily testing of bitterness in beer and astringency in wine (Guinard, 1985).

REFERENCES

Aust, L.B. 1984. Computers as an aid in discrimination testing. Food Technol. **38**(9): 71.
Brady, P.L. 1984. Computers in sensory research. Food Technol. **38**(9): 81.

A Research Note

Ripening Changes in a Blue-Mold Surface Ripened Cheese from Goat's Milk

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————————— ABSTRACT —————————

Samples of blue-mold surface ripened goat milk cheese were ana-lyzed for fat and protein breakdown during ripening. After 2 wk, cheese had a pH of 4.96 and 9.13 micromoles of free fatty acids (F.F.A.)/g fat. After 6 wk, the pH increased to 6.43 and F.F.A. were 160.85 micromoles/g fat. At this time, the cheese rind pH was 7.18 with 253.81 micromoles FFA/g fat. Water soluble protein in the cheese increased from 20.47% at 2 wk of ripening to 26.62% after 4 wk of ripening.

INTRODUCTION

IN THE UNITED STATES, there is greater recognition of goat's milk and its products as acceptable alternatives to cow's milk and products (Haenleim, 1980). In surface mold-ripened cheese, water soluble lipases split fat and the fatty acids produced contribute to the characteristic flavor (Stadhouders and Mulder, 1959). Lipolysis is particularly important for goat's milk cheese, since proportion of short chain fatty acids in goat's milk is approximately twice as great as in cow's milk (Lowenstein et al., 1980). The present study pertains to production of total free fatty acids (FFA), pH changes and protein breakdown in a goat's cheese surface-ripened by *Penicillium roqueforti.*

MATERIALS & METHODS

Milk

Refrigerated raw goat's milk was obtained from mixed herds of goat farms in a one-hundred mile radius of Lansing, Michigan. Upon receipt of milk quality tests such as acidity, pH and fat content were carried out.

Cheese making

No fat standardization of the milk was done. The milk was pasteurized at 65°C with a holding period of 30 min. After cooling to 34°C, 0.014% of direct vat-set lactic culture and 0.028% of single strength rennet were added and stirred for 2-3 min. The curd was cut with 1 cm horizontal and vertical knives, and the curd cubes were gently stirred. After about 30 min of continuous stirring, the whey was partly drained and the curd was dipped into round stainless steel molds (11 cm in diameter) and turned five times at 20 min intervals. The cheese was left draining overnight at 23°C and salted for 90 min in a 20% salt solution. The cheeses were than placed in the cold room, sprayed with an aqueous suspension of *Penicillium roqueforti* spores, and ripened at 8°C and 90-95% relative humidity for 3 wk. A round shaped cheese, 11 cm in diameter and 2.5 cm in height was obtained. Samples of cheese were taken for analysis after 2, 4, and 6 wk of ripening. Five separate batches of cheese (10 gal. of milk each) were made.

Analytical procedures

Whey samples were taken after cutting curd in the cheese vat. Samples of cheese taken at different periods of ripening were ground and thoroughly mixed before each determination. All analyses were made in duplicate.

Titratable acidity was obtained by titrating 10 mL sample (whey or milk) with 0.1N NaOH. pH was measured with a CHEMITRIX Type 60-A digital pH/mv meter equipped with a combination elec-trode. Fat content was determined by the Roese-Gottlieb method with Mojonnier modifications, and the moisture content was deter-mined by the modified Mojonnier method (Milk Industry Founda-tion, 1959). Salt content was determined according to the AOAC (1975) and the density of milk and whey were determined with a Quevenne Lactometer. Total protein and soluble protein were determined according to AOAC (1975) and Kosikowski (1977), respectively. Free fatty acid titer was determined according to Harper et al., (1956).

RESULTS & DISCUSSION

WHILE SEVERAL of the articles relating to the manufac-ture of goat's cheese do give definitive data on the composi-tion of the final product, most do not (Lowenstein et al., 1980). In Table 1, the average composition of this blue-mold ripened cheese is shown. The composition is related to the fresh cheese.

The pH at the time of spraying the mold has a significant effect on mold growth and its lipolytic action on the curd during ripening period. A low pH is desirable, as well as a higher degree of humidity in the cheese. The pH (4.84) and moisture (50.30%) were both satisfactory for the mold growth in this study. The composition (Table 1) resembles the composition of the Chabichou goat cheese (Furtado, 1980).

Important changes during the ripening are summarized in Table 2. A net accumulation of free fatty acids (FFA) was observed. A considerable difference in fatty acid con-tent in different areas of cheese was observed. The FFA content was 253.81 and 17.00 micromoles/g fat in the rind and center of cheese, respectively. After 4 wk, cheese de-veloped an excellent mold growth and color, with a creamy body and smooth texture. The interior of goat's cheese is described as characteristically white with a strong flavor, and typical aroma due to fat composition of goat's milk (Mocquot and Bejambes, 1960).

Changes in pH paralleled lipolytic activity in the goat's cheese (Table 2). Water soluble protein increased from 20.47% at 2 wk of ripening to 26.62% after 4 wk of ripen-

Table 1—Fresh cheese composition[a]

Component	Average
pH	4.84% ± 0.19
Moisture	50.30% ± 2.04
Total solids	42.70% ± 2.04
Fat	21.47% ± 1.51
Fat in dry matter (FDM)	50.28% ± 1.61
Total protein	16.84% ± 0.99
Sodium chloride	1.77% ± 0.14

[a] Average from five lots of cheese

Table 2—*Changes in pH and FFA production during ripening*[a]

Weeks	pH	FFA (μmoles/g of fat)	Soluble protein/ Total protein (%)
0	4.84 ± 0.19	—	—
2	4.96 ± 0.16	9.12 ± 3.32	20.47 ± 3.08
4	5.79 ± 0.29	98.83 ± 17.32	26.62 ± 1.09
6	6.43 ± 0.19	160.85 ± 11.55	—
6 (rind)	7.18 ± 0.12	253.81 ± 31.51	—
6 (center)	5.53 ± 0.46	17.00 ± 6.08	—

[a] Average from five lots of cheese

ing. Up to 36% of soluble protein in Chabichou cheese ripened with *P. roqueforti* has been reported (Wolfschoon and Furtado, 1979). There is a relationship between the increase in the soluble protein and rise of pH during the ripening. During the period in which soluble protein increased from 20.47 to 26.62%, the pH increased by 0.83 unit. It was reported that pH increased from 5.20 to 6.82 in a Chabichou cheese in 30 days of ripening (Wolfschoon and Furtado, 1979). Apparently, the observed increase in soluble protein is related to proteolytic activity of *P. roqueforti* and the amino acids, peptides and amines formed. Presence of two or more proteases in *P. roqueforti* mycelium has been reported. Extracellular protease has an optimum pH of about 5.5, but the intracellular protease has a wider range [5.5-7.0] (Harte, 1974).

The pH and amount of FFA in the rind of cheese are much higher than in its center (Table 2). Since the mold growth proceeded in the cheese surface, the proteolytic and lipolytic activities penetrated in a centripetal way. Protein breakdown and related decrease in the acidity may explain why pH is higher in the rind than in the center of cheese. We observed a change in cheese color following these transformations. In the region of higher lipolysis, near the rind, the cheese was yellowish, whereas in the center it was quite white. After 8-10 days of ripening, the cheese was covered with a thick layer of *P. roqueforti* imparting a dark green color to the cheese rind. After 4 wk of ripening, the cheese possessed an intense aroma and was ready for consumption. The average yield of the cheese was 1.0 kg per 5.54L of goat's milk.

REFERENCES

AOAC. 1975. "Official Methods of Analysis." Association of Official Analytical Chemists, Washington, DC.
Furtado, M.M. 1980. "Fabricacao de Queijo de Leite de Cabra," 1st ed. Levraria Nobel S.A., Sao Paulo.
Haenlein, G.F.W. 1980. Status of world literature on dairy goats, Introductory remarks. J. Dairy Sci. 63: 1591.
Harper, W.J., Schwartz, D.P., and El-Hagarwy, I.S. 1956. A rapid silica-gel method for measuring total fatty acids. J. Dairy Sci. 39: 46.
Harte, B.R. 1974. The effects of process parameters on the formation of volatile acids and free fatty acids in quick ripened blue cheese. M.S. thesis, Library, Michigan State Univ., East Lansing, MI.
Kosikowski, F.V. 1977. "Cheese and Fermented Milk Foods." Edwards Bros., Inc., Ann Arbor MI.
Lowenstein, M., Stephen, J.S., Harold, M.B., and Frank, J.F. 1980. Research on goat milk: A review. J. Dairy Sci. 63: 1631.
Milk Industry Foundation. 1959. "Laboratory Manual. Methods of Analysis of Milk and Its Products." Washington, DC.
Mocquot, G. and Bejambes, M. 1960. Technology of ewe's milk and goat's milk products. Dairy Sci. Abs. 22(1): 1.
Stadhouders, J. and Mulder, H. 1959. Fat hydrolysis and cheese flavor. 3. Surface organisms associated with fat hydrolysis in cheese. The Netherlands Milk & Dairy J. 13: 291.
Wolfschoon, A.F. and Furtado, M.M. 1979. Changes in soluble nitrogen, pH and lactic acid during ripening of Chabichou-type cheese. J. Food Protection 42: 666.
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Duffield, A.M. and Stagg, J.J. 1983. Use of micro-computer with card reader system for flavour profile data handling and storage. In "Sensory Quality in Foods and Beverages. Definition, Measurement and Control," (Ed.) A.A. Williams and R.K. Arkin, p. 115. Ellis Horwood, Ltd., Chichester.
Guinard, J-X. 1985. Computerized time-intensity measurements of bitterness in beer and astringency in wine. M.S. thesis, Univ. of California, Davis.
Jellinek, G. 1964. Introduction to and critical review of modern methods of sensory analysis (Odor, taste and flavor evaluation) with special emphasis on descriptive analysis (Flavor Profile Method). J. Nutr. and Diet. 1(3): 219.
Larson-Powers, N. and Pangborn, R.M. 1978. Paired comparison and time-intensity measurements of the sensory properties of beverages and gelatins containing sucrose or synthetic sweeteners. J. Food Sci. 43(1): 41.
McLellan, M.R. 1984. Application of microcomputer technology in flavor research. Sensory evaluation. In "Computers in Flavor and Fragrance Research," (Ed.) C.B. Warren and J.P. Walradt, p. 1. ACS Symposium Series 261, American Chemical Society, Washington, DC.
McLellan, M.R. and Cash, J.N. 1983. Computerized sensory evaluation: A prototype data-collection system. Food Technol. 37(1): 97.
Neilson, A.J. 1957. Time-intensity studies. Drug and Cosmetic Industry 80(4): 452.
Pecore, S.D. 1984. Computer assisted consumer testing. Food Technol. 38(9): 78.
Savoca, M.R. 1984. Computer applications in descriptive testing. Food Technol. 38(9): 74.
Schmitt, D.J., Thompson, L.J., Malek, D.R., and Munroe, J.H. 1984. An improved method for evaluating time-intensity data. J. Food Sci. 49(2): 539.
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A Research Note
Effects of Food Gums on Zinc and Iron Solubility following *in vitro* Digestion

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─────── ABSTRACT ───────

The effects of three levels (0.1, 0.3 and 0.5%) of locust bean gum, guar gum and sodium alginate on zinc and iron availability were evaluated in milk and soy systems. Availability was estimated as metal solubility after digestion with pepsin-HCl followed by addition of bicarbonate and digestion with pancreatic and bile extracts. Locust bean gum (0.5%) and guar gum (0.5%) reduced zinc solubility by 23.5 and 69.6%, respectively, in the milk and by 41.1 and 40.1% in the soy. Smaller reductions were observed at lower gum levels. The two gums also caused significant reductions in iron solubility from soy. Sodium alginate exerted little effect on mineral solubility.

INTRODUCTION

FOOD GUMS and related polysaccharides are extensively used as gelling, stabilizing, texturizing and emulsifying agents in food processing (Sanderson, 1981). Several of these gums have been found to depress iron absorption in rats (Harmuth-Hoene and Schelenz, 1980; Wolbling et al., 1980). In contrast, Berner and Hood (1983) found that, although sodium alginate bound significant amounts of iron, the iron-alginate complexes were disrupted during an *in vitro* digestion. However, the *in vitro* effects of other food gums on iron bioavailability, as well as the effects of these gums on zinc availability, have not yet been investigated. Consequently, the objectives of the present study were to evaluate the effects of ionic (sodium alginate) and uncharged (guar and locust bean) gums on zinc and iron solubility following *in vitro* gastrointestinal digestions of soy protein and milk.

MATERIALS & METHODS

TUBES CONTAINING 5g of either skim milk or soy protein concentrate and 0.0, 0.1, 0.3 or 0.5% gum (guar gum, locust bean gum or sodium alginate) were subjected to a previously described *in vitro* digestion (Zemel, 1984). The method is a modification of that described by Miller et al. (1981) for determination of iron availability. Samples were first digested with pepsin and hydrochloric acid for 1.5 hr in a 37°C shaking water bath. The pH of each tube was then raised to 7.3 ± 0.1 with 1.0N sodium bicarbonate; a suspension containing bile and pancreatic extracts in 0.1N sodium bicarbonate was then added to each tube. Tubes were sealed to prevent carbon dioxide loss and pH changes and then incubated for an additional 1.5 hr. Immediately following this digestion, samples were centrifuged at 3000 x *g* for 30 min, and the supernatants were re-centrifuged at 25000 x *g* for 1 hr. The supernatants from the second centrifugation were then analyzed for zinc (milk and soy supernatants) and iron (soy supernatants only) by atomic absorption spectrophotometry. Soluble iron was not measured in the milk digests as the very low levels of iron in milk are not nutritionally significant.

Eight replicates of each treatment were performed, and data were evaluated by two way (gum x gum level) analysis of variance.

RESULTS & DISCUSSION

THE RESULTS are summarized in Table 1. All three gums uniformly caused greater decreases in zinc solubility from

soy than from milk. The ionic gum, sodium alginate, was without significant effect on soluble zinc levels in the milk and caused slight reductions in the soy system. In contrast, the two uncharged seed gums, guar and locust bean gums, caused significant reductions in soluble zinc levels in both the milk and the soy digests. In the milk system, guar gum exerted a greater effect than locust bean gum at all three concentrations; this difference was most pronounced at the 0.5% gum level, at which locust bean and guar gums reduced soluble zinc levels by 23.5 and 69.6%, respectively. In the soy system, however, guar gum exerted a significantly ($p < 0.05$) greater effect only at the 0.1 and 0.3% levels. Locust bean gum caused 20.5, 23.6 and 41.4% decreases in soluble zinc at the 0.1, 0.3 and 0.5% levels, while guar gum caused respective decreases of 28.0, 37.0 and 40.1%.

Soluble iron in the soy digests followed a pattern similar to that found with zinc. Sodium alginate was without significant ($p > 0.05$) effect on iron solubility, but both seed gums caused significant ($p < 0.05$) reductions. Again, guar gum exerted a significantly greater effect than locust bean gum. Iron solubility was reduced by 33.9% with the addition of 0.5% locust bean gum, and by 60.7% with the guar gum.

The results of this study demonstrate that two uncharged food gums, guar and locust bean gums, exert substantial negative effects on zinc and iron solubility from soy and on zinc solubility from milk, while sodium alginate

Table 1—Effects of food gums on zinc and iron solubility following in vitro *peptic-pancreatic digestions of milk or soy*

Treatment	Soluble zinc	Soluble iron
	% of Control	
Soy control	100.0 ± 4.5ᵃ	100.0 ± 4.2ᵃ
+ 0.1% L8Gʰ	79.6 ± 6.1ᵈ	98.2 ± 2.1ᵃ
+ 0.3% LBG	76.4 ± 5.8ᵈ	77.3 ± 4.0ᵇ
+ 0.5% LBG	58.6 ± 2.3ᵍ	66.1 ± 5.9ᶜ
+ 0.1% guar	72.0 ± 4.4ᶜ	63.9 ± 3.5ᶜ
+ 0.3% guar	63.0 ± 1.7ᶠ	62.5 ± 4.7ᶜ
+ 0.5% guar	59.9 ± 2.2ᵍ	39.3 ± 8.3ᵈ
+ 0.1% alginate	93.4 ± 1.4ᵇ	99.6 ± 7.2ᵃ
+ 0.3% alginate	83.6 ± 4.9ᶜ	96.4 ± 4.8ᵃ
+ 0.5% alginate	86.7 ± 1.2ᶜ	100.0 ± 1.5ᵃ
Milk control	100.0 ± 4.8ᵃ	—
+ 0.1% LBG	97.9 ± 1.0ᵃ	—
+ 0.3% LBG	78.3 ± 1.5ᵇ	—
+ 0.5% LBG	76.5 ± 1.9ᵇ	—
+ 0.1% guar	79.1 ± 0.8ᵇ	—
+ 0.3% guar	59.2 ± 1.6ᶜ	—
+ 0.5% guar	30.4 ± 4.6ᵈ	—
+ 0.1% alginate	96.9 ± 1.7ᵃ	—
+ 0.3% alginate	94.8 ± 2.5ᵃ	—
+ 0.5% alginate	97.1 ± 6.3ᵃ	—

[a-g] All data are expressed as mean ± standard deviation. Nonmatching superscripts in each column denote significant differences ($p < 0.05$).
[h] LBG = locust bean gum.

A Research Note
Volatile Components of African Mango

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──────── ABSTRACT ────────

Volatile components of African mango were isolated by vacuum distillation and fractionation on silica gel or by trapping on Tenax GC. Analysis by combined GC-MS led to several compounds identified for the first time. Presence in the extract of isolongifolene, eremophilene (17%), and components related to bicyclogermacrene showed the variability of the volatile fraction of mango varieties. Some of these compounds, as well as Δ-3 carene, may be typical of local varieties. The African mango studied is also characterized by presence of free acids: acetic (0.7%), butyric (6%), and hexanoic (5%); and ethyl 3-hydroxybutyrate (5%). These compounds are part of the contribution of lipid metabolism to aroma of mango fruit.

INTRODUCTION

NUMEROUS STUDIES on the volatile components of tropical fruits have appeared in recent years because of their attractive flavor fo consumers. Among these fruits, the flavor of mango (*Mangifera indica* L.), the second most important tropical fruit after bananas, has been extensively investigated (Angelini et al., 1973, Hunter et al., 1974; Gholap and Bandyopadhyay, 1975, 1977; Diaz, 1980; Abd El Baki et al., 1981; Mac Leod and de Troconis, 1982; Idstein and Schreier, 1983; Engel and Tressl, 1983). The results obtained by most of these authors show that considerable differences occur between varieties grown in the same country to the same stage of ripeness. Most of the works reported to date have been undertaken using mango cultivated in India, Egypt or South and Central America; No study concerning African mango has appeared in the literature. The present work was undertaken to investigate the flavor components of mango, and the differences in composition between varieties.

MATERIALS & METHODS

Mangos

Mango fruits used in this study were obtained from ungrafted trees growing at Pout and Sebikhotane, Senegal. Ninety kilograms of fruits in the full ripe stage were washed, crushed and refined (screen 1 mm in diameter) using a pilot pulper-refiner-pitter. The puree thus obtained (59 kg) was homogenized, poured in polyethylene bags without any heat treatment and frozen at −20°C until use (5 − 6 months). All these operations were performed in the pilot plant of Institut de Technologie Alimentaire de Dakar.

Sampling techniques

Adsorption on Tenax GC. The trapping technique described by Jennings et al. (1972) was used.
Vacuum steam distillation. The volatile components were obtained by stripping according to Siesso and Crouzet (1977).

Silica gel fractionation

The mango extract was fractionated by the method of Palmer (1973) into four fractions (I to IV).

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Analytical gas chromatography

A Varian Aerograph 2400 gas chromatograph fitted with FID and a 60 m × 0.5 mm glass capillary column coated with Carbowax 20 M was used. The temperature was held at 70°C for 10 min, then programmed at 2°C/min to 180°C. The carrier gas was nitrogen at 7 mL/min. The output signal was fed through a Spectra Physics SP 4000 Central processor and plotted on a SP 4050 printer plotter. Authentic samples of chemicals used for identification purpose based on retention determination were obtained from commercial supply houses or were received as gifts. Muhuhu and Copahu essential oils were used for identification of some sesquiterpenic hydrocarbons. Kovats index given by Jennings and Shibamoto (1980) were used.

Gas chromatography-Mass spectrometry

An LKB 2091 mass spectrometer was coupled with the glass capillary column used for analytical gas chromatography and operated under the conditions previously indicated. The ionizing voltage was 70 eV and source temperature 230°C.

RESULTS & DISCUSSION

THE COMPOUNDS IDENTIFIED in the African mango concentrate obtained by vacuum steam distillation are listed in Table 1. Some of them — camphene, ethyl styrene, isolongifolene, α-bergamotene, aromadendrene, α- and δ-guaiene, eremophilene, alloaromadendrene, α-muurolene, butyric and hexanoic acids, benzyl and furfuryl alcohols, 2-acetyl pyrrole and dihydroactinidiolide — are reported for the first time as mango volatile components.

Particular attention must be paid to terpenic and sesquiterpenic hydrocarbons. As stated by several authors (Mac Leod and Gonzalez de Troconis, 1982; Engel and Tressl, 1983) these compounds were quantitatively the most important (about 40% of the sample); furthermore, the volatiles trapped on Tenax GC (Table 2) were essentially compounds with a terpenic skeleton. As the mixture obtained after desorption of the porous polymer had an aroma reminiscent of mango we can assume that these compounds are important contributors to this aroma. Δ-3 Carene, previously isolated from Venezuelan mango fruit (Mac Leod and Gonzalez de Troconis 1982) and described by these authors as having an aroma of mango leaves, was also present in the concentrate obtained from the African mango under study. More recently Gholap and Bandyopadhyay (1984) found that two compounds tentatively identified as car-3-ene and cis-ocimene contributed to the mango odor of the rhizome of *Curcuma amada* Roxb. Both extracts, obtained after vacuum distillation and adsorption on Tenax GC, were characterized by presence of considerable quantities (17% and 30%, respectively), of a sesquiterpenic hydrocarbon identified as eremophilene. Although we are not in possession of an authentic sample of this product its mass spectrum matched that of the spectrum of the authentic product published elsewhere (Stenhagen et al., 1974). This compound, isolated from Valerian oil (Witek and Krepinsky, 1966) or from rhizomes of plants of genus *Petasites* (Hochmannova et al. 1962), has not been described previously as a fruit volatile component. Isolongifolene was present in the two extracts obtained by vacuum distillation and adsorption on Tenax GC and is also

Table 1—Identification of volatile components isolated from African mango

Peak N°	Component	Fraction	Kovats index[a]	Relative abundance %	Evidence[c]
1	ethyl butanoate	II	1025	0,2	MS,RT
2	α-pinene	I	1039	0,1	MS,RT
3	Toluene	I	1055	0,9	MS,RT
4	Camphene[b]	I	1083	0,2	MS,RT
5	hexanal	II	1084	tr	MS,RT
6	β-pinene	I	1124	0,1	MS,RT
7	myrcene	I	1156	1,2	MS,RT
8	Δ3-carene	I	1165	3,6	MS,RT
9	α-phellandrene	I	1177	0,1	MS,RT
10	limonene	I	1206	1,6	MS,RT
11	β-phellandrene	I	1216	0,2	MS
12	cis-ocimene	I	1228	0,3	MS,RT
13	γ-terpinene	I	1251	0,3	MS,RT
14	ethyl-styrene[b]			tr	MS
15	p-cymene	I	1272	3,5	MS,RT
16	allocimene	I		0,2	MS
17	acetoine	III	1276	0,1	MS,RT
18	dimethylstyrene	IV		trace	MS
19	terpinolene	I	1287	1,5	MS
29	hexanol	III	1316	2,0	MS,RT
21	(Z)-3-hexene-1-ol	III	1351	1,5	MS,RT
22	(E)-2-hexene-1-ol	III	1368	0,2	MS,RT
23	acetic acid	III	1419	0,7	MS,RT
24	butyl hexanoate	II	1402	0,3	MS
25	ethyl octanoate + cis-linalooloxide	II	1423	0,8	MS,RT
26	furfural		1449	0,2	MS,RT
27	trans-linalool oxide	II	1451	tr	MS,RT
28	2-acetyl furan		1491	0,1	MS,RT
29	benzaldehyde	II	1502	tr	MS,RT
30	linalool	III	1506	1,0	MS,RT
31	unknown			1,5	
32	isolongifolene[b]	I		1,2	MS,RT
33	α-copaene	I	1519	0,9	MS,RT
34	5-methyl furfural		1563	0,6	MS,RT

Table 1 continued

35	ethyl 3-hydroxy-butanoate	II		5,2	MS
36	α-bergamotene[b]	I		1,4	MS,RT
37	α-gurjunene	I		1,5	MS
38	butyric acid[b]	III		6,1	MS,RT
39	β-caryophyllene	I	1618	0,2	MS,RT
40	aromadendrene[b]	I	—	0,2	MS,RT
41	ethyl decanoate	II	1624	0,1	MS,RT
42	α-guaiene[b]	I	1629	0,1	MS
43	acetophenone		1627	trace	MS,RT
44	γ-butyrolactone	III	1632	0,6	MS,RT
45	furfuryl alcohol[b]			0,6	MS,RT
46	α-humulene	I	1632	2,6	MS
47	aromatic component			0.8	MS
48	α-terpineol	III	1661	0,4	MS,RT
49	sesquiterpene			0,5	
50	eremophilene[b]	I		17,5	MS
51	alloaromadendrene[b]	I	1662	1,6	MS,RT
52	δ-guaiene[b]	I		0,5	MS
53	sesquiterpene	I		0,5	MS
54	geranial	II	1730	0,7	MS,RT
55	γ-hexalactone	IV		1,1	MS,RT
56	α-muurolene[b]	I	1730	1,1	MS,RT
57	δ-cadinene	I	1761	0,8	MS,RT
58	γ-cadinene	I	1766	0,3	MS,RT
59	benzyl alcohol[b]	II	1822	0,1	MS,RT
60	damascenone			0,1	MS,RT
61	hexanoic acid[b]	IV		5,1	MS,RT
62	ethyl dodecanoate	II	1826	3,0	MS,RT
63	2-phenyl ethanol	III	1855	0,7	MS,RT
64	γ-octalactone	IV	1883	2,7	MS,RT
65	β-ionone		1918	0,6	MS,RT
66	2-acetyl pyrrole[b]		1935	0,2	MS,RT
67	γ-nonalactone	IV	1991	3,0	MS,RT
68	ethyl tetradecanoate	II	2027	0,3	MS,RT
69	γ-decalactone	IV	2101	0,4	MS,RT
70	δ-decalactone	IV	2144	0,6	MS,RT
71	dihydroactinidiolide[b]		2324	trace	MS,RT
72	ethyl hexadecanoate	II	—	0,6	MS,RT

[a] Jennings and Shibamoto (1980).
[b] Identified for the first time in mango volatile components.
[c] MS mass spectrum; RT = retention time.

identified for the first time in mango volatile components, and more generally, in fruit aroma components. However, this compound may be an artefact formed from longifolene (Ramdas Nayak and Dev, 1960). The presence of these three compounds, as well as the identification of α- and δ-guaiene and α-muurolene in some mango varieties, illustrates the variability underlined by several authors.

Among the African mango volatile components we found aromadendrene and alloaromadendrene besides α-gurjunene, previously identified by Engel and Tressl (1983) in variety Baladi. According to Tressl et al. (1983) these tricyclic sesquiterpenes may arise from bicyclogermacrene and are possible precursors of sesquiterpenic alcohols: ledol, veridiflorol . . . through epoxides.

The presence of ledol (Diaz, 1980) and of bicyclogermacrene, globulol and viridiflorol stated by Engel and Tressl (1983) makes this sequence possible in mango fruit.

Mac Leod and Gonzalez de Troconis (1983) pointed out the presence of a dimethyl styrene among the volatile components of mango and presumed that this compound contributed to fresh mango fruit flavor. A dimethyl styrene and one isomer, ethyl styrene were identified on the basis of their mass spectra (Stenhagen et al., 1974). Though α-p dimethyl styrene is known to arise from citral by cyclisation (Kimura et al., 1983) no increase in the concentration of styrene derivatives occured during heat treatment of mango juice (Sakho et al., 1984). Thus we can assume that the two styrenes found are really present in mango fruit.

Organic acids found in the present work may be present in the fruit or originate from the hydrolysis of esters during

Table 2—Volatile components of African mango identified after trapping on Tenax GC

Peak no.	Component	Peak no.	Component
1	α-pinene	11	ethyl octanoate
2	toluene	12	Isolongifolene
3	camphene	13	α-copaene
4	hexanal	14	α-bergamotene
5	β-pinene	15	α-gurjunene
6	Δ3-carene	16	β-caryophyllene
7	limonene	17	eremophilene
8	γ-terpinene	18	alloaromadendrene
9	p-cymene	19	α-muurolene
10	allocimene		

crushing or extraction step. The presence in our extract of some esters previously identified in mango (ethyl esters of fatty acids, ethyl butanoate, butyl hexanoate and ethyl-3-hydroxybutanoate) shows that hydrolysis does not occur during sample preparation. Ethyl-3-hydroxybutanoate (ca. 5% of the extract) may be considered as a characteristic compound of the variety studied, as well as of the variety Baladi. Conversely, it is less important in variety Alphonso (Engel and Tressl 1983). The presence of acids, esters, and also lactones agrees with previous works concerning the contribution of lipid metabolism in the development of the

aroma and flavor of mango fruit during ripening (Bandyo-padhyay and Gholap, 1973; Gholap and Bandyopadhyay, 1980).

C_6 aldehydes and alcohols previously detected in variety Alphonso were also present in aroma extract from African mango. However, these compounds formed during crushing of most of the fruits (Ericksson, 1979) are dependent on the enzymatic equipment of the fruit and conditions of crushing (Kazeniac and Hall, 1970).

Some of the compounds isolated — furan derivatives, acetyl pyrrole, β-ionone, damascenone, dihydroactinidiolide, linalool oxides — are clearly artefacts formed during preparation of pulp or during extraction step.

REFERENCES

Abd El-Baki, M.M., Askar, A., El Samahy, S.K., and Abd El-Fadeel, M.G. 1981. Studies on mango flavor. Deuts. Lebensm. Rund. 77: 139.

Angelini, P., Bandyopadhyay, C., Rao, B.Y.K., Gholap, A.S., and Bazinet, M.L. 1973. Studies on aroma of ripe mango pulp: identification of aroma bearing compounds 33rd Annual meeting of the Institute of Food Technologists, Miami Beach, FL.

Bandyopadhyay, C. and Gholap, A.S. 1973. Relationship of Aroma and flavour characteristics of mango (Mangifera indica L.) to fatty acid composition. J. Sci. Food Agric. 24: 1497.

Diaz, N. 1980. A preliminary study on the flavor and aroma components of four mango varieties. J. Agric. Univ. P.R. 64: 357.

Engel, K.H. and Tressl, R. 1983. Studies on the volatile components of two mango varieties. J. Agric. Food Chem. 31: 796.

Ericksson, C.E. 1979. Review of biosynthesis of volatiles in fruits and vegetables since 1975 in Progress in flavour research. p. 159. Applied Sci. Publ. Ltd., London.

Gholap, A.S. and Bandyopadhyay, C. 1980. Fatty acid biogenesis in ripening Mango (Mangifera indica L. var Alphonso.). J. Agric. Food Chem. 28: 839.

Gholap, A.S. and Bandyopadhyay, C. 1984. Characterization of green aroma of Raw mango (Mangifera indica L.). J. Sci. Food Agric. 28: 885.

Gholap, A'S. and Bandyopadhyay, C. 1980. Fatty acid biogenesis in ripening Mango (Mangifera indica L. var Alphonso.). J. Agric. Food Chem. 28: 839.

Gholap, A.S. and Bandyopadhyay, C. 1984. Characterization of

mango-like aroma in Curcuma amada Roxb. J. Agric. Food Chem. 32: 57.

Hochmannova, J. Novotny, L., and Herout, V. 1962. Hydrocarbons from Petasites albus rhizomes; Sesquiterpenic hydrocarbons from rhizomes of Petasites officinalis. Collect. Czech. Chem. Commun., 27: 1711; 1870.

Hunter, G.L.K., Bucek, W.A., and Radford, T. 1974. Volatile components of canned Alphonso mango. J. Food Sci. 39: 900.

Idstein, H. and Schreier, P. 1983. Change of volatiles during maceration and liquefaction of selected tropical fruits. Euro Food Chemistry II, Roma.

Jennings, W.G., Wohleb, R., and Lewis, M.J. 1972. Gas chromatographic analysis of headspace volatiles of alcoholic beverages. J. Food Sci. 37: 69.

Jennings, W.G. and Shibamoto, T. 1980. "Qualitative Analysis of Flavor and Fragrance Volatile by Glass Capillary Gas Chromatography. Academic Press, New York.

Kazeniac, S.J. and Hall, R.M. 1970. Flavor chemistry of tomato volatile. J. Food Sci. 35: 519.

Kimura, K., Nishimura, H., Iwata, I., and Mizutani, J. 1983. Deterioration mechanism of lemon flavor. 2 Formation of off-odor substances arising from citral. J. Agric. Food Chem. 31: 801.

Mac Leod, A.J. and Gonzalez de Troconis, N. 1982. Volatile flavour components of mango fruit. Phytochemistry 21: 2523.

Palmer, J.K. 1973. Separation of components of aroma concentrates on the basis of functinal group and aroma quality. J. Agric. Food Chem. 21: 923.

Ramdas Nayak, V. and Dev, S. 1960. Sesquiterpenes: hydration of longifolene Tetrahedron 8: 42.

Sakho, M., Crouzet, J., and Seck, S. 1984. Changes of mango volatile components during heating. Lebensm. Wissen. Tech. (In press).

Siesso, V. and Crouzet, J. 1977. Tomato volatile components: effects of processing. Food Chem. 2: 241.

Stenhagen, E., Abrahamson, S., and Mac Lafferty, F.W. 1974. "Registry of Mass Spectral Data." John Wiley & Sons Inc., New York.

Tressl, R., Engel, K.H., Kossa, M., and Köpplu, H. 1983. Characterization of tricyclic sesquiterpenes in hop (Humulus Lupulus, var Hersbrucker spät). J. Agric. Food Chem. 31: 892.

Witek, S. and Krepinsky, J. 1966. The composition of valerian oil (Valeriana officinalis) Collect. Czech. Chem. Commun. 31: 1113.

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exerts little significant effect under conditions which simulate the use of these gums in food systems. The *in vitro* procedure used in this study simulates only luminal occurrences in the gut without considering mucosal control of absorption; however, any decreases in mineral solubility may be considered to predict decreases in bioavailability, as solubility is a prerequisite to absorption. Although sodium alginate did cause a slight, significant decrease in zinc solubility following the digestion of the soy, iron solubility was unaffected. Similarly, Berner and Hood (1983) reported that, although alginate binds iron, alginate-iron complexes are disrupted during an *in vitro* digestion. In contrast, zinc and iron complexes with the two seed gums (guar and locust bean) used in the present study do not appear to be easily disrupted, as these gums caused substantial decreases in zinc and iron solubility. The reason for this difference is not clear, as the nature of metal binding to these uncharged gums is unexplained. It is possible that, in the alkaline environment of the small intestine, hydroxylic protons are released from the gums, allowing the formation

of metal-oxygen bonds. This phenomenon has been demonstrated with other neutral polysaccharides, such as cellulose, in an alkaline environment (Rendleman, 1978).

REFERENCES

Berner, L.A. and Hood, L.F. 1983. Iron binding by sodium alginate. J. Food Sci. 48: 755.

Harmuth-Hoene, A. and Schelenz, R. 1980. Effect of dietary fiber on mineral absorption in growing rats. J. Nutr. 110: 1774.

Miller, D.D., Schricker, B.R., Rasmussen, R.R., and VanCampen, D.R. 1981. An in vitro method for estimation of iron availability from meals. Am. J. Clin. Nutr. 34: 2248.

Rendleman, J.A. 1978. Metal-polysaccharide complexes—part 2. Food Chem. 3: 127.

Sanderson, G.R. 1981. Polysaccharides in foods. Food Tech. 35(7): 50.

Wolbling, R.H., Becker, G., and Froth, W. 1980. Inhibition of the intestinal absorption of iron by sodium alginate and guar gum in rats. Digestion 20: 403.

Zemel, M.B. 1984. In Vitro evaluation of the effects of orthotripoly- and hexametaphosphate on zinc, iron and calcium bioavailability. J. Food Sci. 49(6): 1562.

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A Research Note

Effect of Methionine Supplementation on Nitrogen Balance in Infants Fed a Low-Cost Soy-Oats Formula

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─────── ABSTRACT ───────

A nitrogen balance study, comparing a low-cost soy-oats infant formula with and without supplemental methionine, was carried out with infants of ages 3 - 26 months at a single equivalent level of nitrogen intake (approximately 850 mg/kg/day). Nitrogen retention was found to be unaffected by methionine supplementation. Comparison of data obtained in this study with previous research revealed that a critical value for intake of total L-sulfur amino acids apparently exists in infants 3 - 26 months of age, below which methionine supplementation will increase nitrogen retention, but above which it will not. This value appears to coincide with that of 49 mg/kg/day recommended for infants by the FNB.

INTRODUCTION

SOME INVESTIGATORS have studied the effect of methionine supplementation of soy-based infant formulas utilizing nitrogen balance techniques. Fomon and Ziegler (1979) investigated the effect of supplementing soybeans with 5 mg of L-methionine per gram of protein with intakes of the order of 2.4g protein/kg/day in infants. It was found that methionine supplementation did not influence nitrogen retention. Graham (1971) reported results of studies in eight children between 6 and 23 months of age who had recently recovered or were in the process of recovering from severe malnutrition. Daily protein and energy intakes were 1.25 - 2.00g and 100 - 150 kcal/kg/day, with 4 - 7% of the total energy derived from protein. Protein sources were a soy-milk based on toasted soy flour with and without a supplement of 11 - 22 mg DL-methionine/kg body weight/day (three children); a soy protein isolate with and without 16 - 20 mg DL-methionine/kg/day (four children); and full-fat soy flour with and without 20 mg DL-methionine/kg/day (one child). Methionine supplementation was found to increase nitrogen retention in all cases.

In a previous work, Del Valle et al. (1981) described the development and evaluation of a powdered low-cost soy-oats infant formula in rats as well as in infants. This formula was supplemented with methionine in order to raise the total sulfur amino acids to levels slightly above those recommended by FAO/WHO (1973) for children. In that paper, data comparing the Protein Efficiency Ratio (PER) and Net Protein Utilization (NPU) of the formula with and without added methionine were reported. It was found that, while absolute PER was significantly increased (p < 0.01) from 2.55 to 2.99, NPU was unaffected by supplementation. Since both PER and NPU were determined with rats, it was desirable to compare the effect of methionine

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supplementation of the formula using human subjects. The purpose of this work, therefore, was to carry out a nitrogen balance study with infants, comparing the formula with and without added methionine.

MATERIALS & METHODS

THE NITROGEN BALANCE STUDY was conducted at Hospital Infantil del Estado de Chihuahua (Matamoros 2201, Chihuahua, Chih., Mexico). Nutritional and other data of the formulas employed were those reported by Del Valle et al. (1981) and are summarized in Table 1, except that they were prepared with or without 0.2% supplemental DL-methionine. Six infants of ages 3 - 26 months were fed the formula with and without methionine at a single level of nitrogen intake according to the method of Hegsted (1974) for a period of 5 days. The technique utilized was described in detail by Del Valle et al. (1981). Growth rates were not determined.

Final data obtained in the study were (formulas with and without methionine): nitrogen intake, mg/kg/day; nitrogen absorbed, mg/kg/day; nitrogen retained, mg/kg/day; nitrogen retained as percent of absorbed; and digestibility, equal to nitrogen absorbed as percent of ingested. Resulting mean values were compared for significant differences by application of t-tests (Snedecor and Cochran, 1967).

Special parameters for this work, as well as for those of Fomon and Ziegler (1979) and Graham (1971), such as formula methionine supplementation, protein intake and supplemented and total methionine intakes mentioned in the following section, were calculated

Table 1—Nutritional and other data of the soy-oats infant formula utilized, not including methionine (Del Valle et al., 1981)

Substance	Content per 100g
PROTEIN	17.5 g
FAT	22.2 g
CARBOHYDRATE	53.1 g
ASH	2.2 g
CRUDE FIBER	1.0 g
MOISTURE	4.0 g
Vitamin A	1800 I.U.
Thiamine	0.45 mg
Riboflavin	0.70 mg
Ascorbic Acid	35.0 mg
Vitamin D	330 I.U.
Folic Acid	25.0 mcg
Pantothenic Acid	2.0 mg
Pyridoxine	0.30 mg
Vitamin E	4.0 I.U.
Vitamin B_{12}	1.0 mcg
Niacin	5.0 mg
Sodium	115 mg
Potassium	524 mg
Calcium	425 mg
Phosphorus	345 mg
Chloride	367 mg
Copper	0.30 mg
Iron	12.6 mg
Zinc	3.0 mg
Iodine	53.4 mcg
Magnesium	50.0 mg

utilizing data given in the same studies as well as from other sources (Del Valle et al., 1981; FAO, 1970).

RESULTS & DISCUSSION

TABLE 2 summarizes data obtained in the nitrogen balance study. Comparison of the corresponding mean values for the formulas with and without methionine revealed that no significant difference existed between them, thus proving that addition of methionine under conditions of the study did not affect nitrogen retention. The significance of these results may be better understood by comparing data obtained in this study with those of Fomon and Ziegler (1979) and Graham (1971). In all formulas supplemented with methionine, added amounts of this substance, expressed as L-methionine, were similar being of the order of 6 mg/g protein. Protein intake, on the other hand, varied widely ranging from 1.25 – 2.00 g/kg/day in the work of Graham (1971) to 5.1 – 5.6 g/kg/day in the present work. As a result, supplemental L-methionine intake, expressed as mg/kg/day reflected the same trend as protein intake.

The most important comparison, however, is probably between total sulfur amino acids (L-form) intake for the different studies. Values of this parameter, expressed as mg/kg/day were as follows: formulas with supplemental methionine: Graham (1971), 34 – 68; Fomon and Ziegler (1979), 65; this study, 235; formulas without supplemental methionine: Graham (1971), 28 – 44; Fomon and Ziegler (1979), 53; this study, 184. This is interesting because nitrogen retention was unaffected by methionine supplementation in this work as well as in that of Fomon and Ziegler (1979) but was increased in the work of Graham (1979). It appears therefore that all other things being equal, nitrogen retention is unaffected by methionine supplementation if total L-sulfur amino acid intake exceeds a certain critical value but will increase if it drops below that value. In the latter case, nitrogen retention increases if total L-sulfur amino acid intake is increased either by consuming more protein or consuming the same amount of protein with supplemental methionine. The critical value for total L-sulfur amino acid intake from the above data appears to lie between 48 and 53 mg/kg/day. This is in

agreement with the requirement of 49 mg/kg/day for total L-sulfur amino acids estimated by the Food and Nutrition Board (FNB, 1975) for infants. This same effect has been noted by Torun et al. (1981) for adults as well as for children.

It could be argued that in the investigation of Graham (1971) nitrogen retention was affected by methionine supplementation because his test subjects had either recently recovered or were in the process of recovering from severe malnutrition and not because of the above suggested effect. If that were the case a similar trend would have been found in this study, where all test subjects were in a similar nutritional status to those of Graham (1971). No such trend was observed.

One final comment may be made with respect to the fact that the total L-sulfur amino acid intake (mg/kg/day) was higher for the soy-oats formula without methionine utilized in this investigation than for the soy formulas supplemented with methionine utilized in the others (Graham, 1971; Fomon and Ziegler, 1979). This was due to two factors: (1) the total L-sulfur amino acids of the soy-oats formula without methionine was higher than for soy alone (Del Valle et al., 1981; FAO, 1970); and (2) the protein intake in this study (mg/kg/day) was considerably higher than in the other two.

SUMMARY & CONCLUSIONS

SUPPLEMENTATION of the soy-oats formula with methionine under conditions of this study did not affect nitrogen retention. Comparing these data with previous results, it appears that the effect of methionine supplementation on nitrogen retention in infants depends upon total L-sulfur amino acid intake: if this is below approximately 49 mg/kg/day, nitrogen retention will increase with methionine supplementation, but if it is above that value, nitrogen retention will be unaffected by added methionine. Total sulfur amino acid intake is determined by content of these substances in ingested protein and also by total protein intake (g/kg/day).

REFERENCES

Del Valle, F.R., Villanueva, H., Reyes-Govea, J., Escobedo, M., Bourges, H., Ponce, J., and Munoz, M.J. 1981. Development, evaluation and industrial production of a powdered soy-oats infant formula using a low-cost extruder. J. Food Sci. 46: 192.
FAO. 1970. Amino-Acid Content of Foods and Biological Data on Proteins. Food and Agricultural Organization of the United Nations, Rome, Italy.
FAO/WHO. 1973. Energy and Protein Requirements, Ad Hoc Expert Committee, World Health Organization Tech. Report, Series No. 522, Rome, Italy.
FNB (Food and Nutrition Board, National Research Council). 1975. Improvement of protein nutriture. National Academy of Sciences, Washington, DC.
Fomon, S.J. and Ziegler, E.E. 1979. In "Soy Protein and Human Nutrition," (Ed.) Wilcke, H.L., Hopkins, D.T., and Waggle, D.H., p. 79. Academic Press, New York.
Graham, S.C. 1971. In "Amino Acid Fortification of Protein Foods," (Ed.) Scrimshaw, N.S. and Altschul, A.M. p. 222. M.I.T. Press, Cambridge, MA.
Hegsted, D.M. 1974. Improvement of protein nutrition. Natl. Res. Council Committee on Amino Acids, National Academy of Sciences, Washington, DC.
Snedecor, G.W. and Cochran, W.G. 1967. "Statistical Methods." The Iowa State University Press, Ames, IA.
Torun, B., Viteri, F.E., and Young, V.R. 1981. Nutritional role of soya protein for humans. J. Am. Oil Chem. Soc. 58(3): 400.
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Table 2—Results of nitrogen balance determination, comparing soy-oats infant formula with and without methionine

Concept[a]	Soy-oats formula with methionine[b,c]	Soy-oats formula without methionine[b,c]
Nitrogen ingested, mg/kg/day	901 ± 312	810 ± 212
Nitrogen absorbed, mg/kg/day	552 ± 275	557 ± 162
Nitrogen retained, mg/kg/day	257 ± 183	302 ± 167
Nitrogen retained, percent of absorbed	43 ± 15	51 ± 20
Digestibility, percent	63 ± 17	69 ± 9

[a] Infants: six, ages 3 – 26 months.
[b] Composition of formula reported in Table 1 (Del Valle et al., 1981).
[c] Results expressed as mean ± standard deviation.