4 July-August 1976
Volume 41 : Number 4
Coden: JFDSAZ 41(4) (729–979) 1976

)URNAL FOOD SCIENCE

SCIENCE and ENGINEERING

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A PUBLICATION OF THE INSTITUTE OF FOOD TECHNOLOGISTS

BASIC SCIENCE

RESEARCH NOTES

July-August 1976
Volume 41 : Number 4

JOURNAL of FOOD SCIENCE

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 JOURNAL of FOOD SCIENCE (formerly *Food Research*) is published six times a year (bimonthly) by Institute of Food Technologists, Suite 2120, 221 N. LaSalle Street, Chicago, Illinois 60601 USA. Printed in USA. Second class postage paid at Chicago, Ill. and at additional mailing offices.

Letters

Physical/Chemical Principles Challenged

I wish again to express my concern over food scientists perpetuating ideas that are not based on sound physical chemical principles. I am referring to the article by Wu et al. (1975) on use of a fluctuating temperature treatment (FTT) for protein or enzyme inactivation. The article implies that there is some magic in cycling the temperature up and down around a certain set temperature with a resultant greater increase in amount of destruction occurring.

Let us examine the studies made. With rennin, the authors placed samples at a high temperature T_1 for 6 min then brought it to T_3 (T_1 – 10°C) for 6 min and back and forth for several cycles (unspecified in the paper). This they compare to heating at constant temperature T_2 which is half way in between (T_1 – 5°C). They imply that this causes a change in activation energy and a faster rate of coagulation occurs.

The former is untrue, while the latter is true but used wrongly. To prove this, one must determine first what is the reaction order. From their Figure 1, they imply that the reaction is not first order. From enzyme kinetics, reactions are either first or zero order. As substrate concentration increases relative to enzyme concentration, the rate increases to a maximum and then remains constant. They show that an increase in E increases the rate at presumably constant (S). Unfortunately as in most enzyme studies, an endpoint analysis is used; that is, the time for coagulation is measured and is used as the rate. Figure 1 proves nothing. If the reaction order with respect to enzyme is to be measured, they should plot log (rate) vs log (E), the slope of which is a straight line which I find to have a value of 1. This proves that the order is 1 with respect to enzyme and zero with respect to substrate, which is meaningful from a kinetic standpoint. Thus, this allows them to use endpoint analysis thereby assuming the rate equals the reciprocal of time to coagulation.

In addition, their data plotted as log time vs 1/T°K give an inactivation energy of about 15,000 cal/mole. It is hard to read off of journal graphs, but this value is typical of enzyme reactions. Based on this the Q_{10} comes from:

$$\log Q_{10} = \frac{2.2\,E_a}{(T)\,(T+10)} \qquad (1)$$

or a value of about Q_{10} equal to 2.24 which is common for enzyme activity. Using this at 40°C vs an experiment at 45°C, one can compare the rates to an FTT experiment with a ± 5°C amplitude. I presume they kept cycling for 6 min intervals until coagulation occurred, and measured time to the endpoint. Thus based on this, for a zero order reaction to reach 100% completion, such as, coagulation;

$$1 = (\text{rate})_{T_1 - 10} \cdot 6 + (\text{rate})_{T_1} \cdot 6 + \ldots .$$

with repetitions until the fractions equal or exceed one. In making these calculations, however, I have had to assume constant temperature at each time. This is not true since there are both come up and come down times, which they do not mention. This is a common fault in most experimental designs. In fact, in the same journal, the paper by Pasch and von Elbe (1975) makes the same error. In the present paper there are no rate data between 40 and 45, but presumably the rate is a maximum near 42–43°C.

Using Figure 2 data for 0.032 mg/ml of enzyme, the following data are obtained:

	(Coagulation Time)		
35°C	θ_s	=	41.7
37°C	θ_s	=	40.0
40°C	θ_s	=	37.0
42°C	θ_s	=	34.5
45°C	θ_s	=	43.5

assuming this to be the maximum. Then → at 40°C CTT experiment, complete reaction equals:

$$\left[\frac{1}{37.0}\right] \cdot 37.0 = 1$$

at 35–40–45° from Figure 2, time = 36.5 min for completion or for 6 min intervals assuming after cooling or heating, and the system must go through the come up and come down times with averages after the initial 6 min of 37 and 42°C.

$$1 = \left[\frac{1}{43.5}\right] 6 + \left[\frac{1}{40}\right] 6 + \left[\frac{1}{34.5}\right] 6 + \left[\frac{1}{40}\right] 6 + \left[\frac{1}{34.5}\right] 6 + \left[\frac{1}{40}\right] 6 + \left[\frac{1}{34.5}\right] \theta$$

$$= 0.138 + 0.150 + 0.174 + 0.150 + 0.174 + 0.150 + \theta \left[\frac{1}{34.5}\right]$$

or for the last cycle the time is about 2.2 min for a total time of 38.2. Comparing this to the 37 min they got, I admittedly find a slightly slower rate, for the fraction of total time consumed to reach complete inactivation is:

$$f = \left[\frac{1}{\theta_{T_1}}\right] \theta_1 + \left[\frac{1}{\theta_{T_2}}\right] \theta_2 + \ldots \left[\frac{1}{\theta_{T_N}}\right] \theta_N \qquad (2)$$

where θ_{T_N} is time to reach coagulation at temperature T_N and

θ_N is time held at T_N

whereas, their Figure 2 shows just a slightly larger rate. This is due to the problem of temperature estimates I made and trying to read values off of a graph. The point is that application of zero order kinetics would have proven the point. The same can be done for the 45° data; however, there the problem is that inactivation is very rapid at 50°C so that the first cycle at 50°C destroys the enzyme and no coagulation occurs. That is why the apparent maximum in rate changes, not as they suppose due to some mystical change in activation energy.

The second part of their paper is treated even poorer, since they are using a ±25°C fluctuation and come up and come down are harder to estimate. Also, the reaction should proceed by a first order mechanism where:

$$-\Sigma(k_1\theta_1 + k_2\theta_2 + \ldots k_N\theta_N)$$

$$C = C_o e \qquad (3)$$

where C = amount left after treatment at temperature T for θ_1 min plus temperature T_2 for θ_2 min, etc. C_0 = initial concentration; and k_N = rate at temperature T_N.

To estimate the rate constant, I presumed 100% activity in the initial sample and used the data of Figure 3 to get the values since:

$$k = \frac{\ln\left[\frac{\%\ \text{Activity}}{100}\right]}{6\ \text{min}} \cdot 1$$

I also estimated a fairly low activation energy of 6,200 cal/mole from their data. Given this, then we compare the 32.5% inactivation at 125°C to treatment B which they state at 125 ± 25°C with a 6 min cycle for 6 min. Thus they really heated at 150°C for 6 min, which gives them the value of 13% which is in close agreement with the results at 150°C in Figure 3 of about 16%. Treatment C using my equation (3) gives 27% inactivation which is very close to their predicted 24.4% and Treatment D gives 24% inactivation (assuming the temperatures never really reach the presumed temperatures). This again is close to the measured values.

My overall point is that by using a good kinetic basis, one could have shown there is no magic in a fluctuating temperature experiment and that it is only due to the Q_{10} effects on the reaction. I want to make this a strong point, otherwise we will introduce a new art into food science and perpetuate an idea which in practice is correct but in explanation needs a sound physical chemistry basis.

—*Theodore P. Labuza,* Dept. of Food Science & Nutrition, University of Minnesota, St. Paul, MN 55108.

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Enzymes Not Pure Chemical Systems

We thank Dr. Labuza for taking the time to evaluate our paper by making calculations of his own. To explain everything on a physical chemical basis would be fine if enzymes were pure chemical systems.

Energy of activation (E_a) is a constant if conditions are constant, but our prior papers and the work of others amply bear out the idea that E_a may be different when different conditions exist. Segel (1975) points this out in showing log k vs 1/T plots and explains changes in the slope arising from a different step becoming rate-limiting. He states (p. 934) that the E_a calculated from the Arrhenius plot will be an apparent or "average" value and that the plot may be nonlinear if different steps become rate-limiting at different temperatures. Q_{10} values certainly change both below and above the optimum temperature for an enzyme, as any enzyme activity curve makes self evident. Both for Q_{10} values and E_a, the ratio for the reaction rates at the two temperatures are involved. If one changes, the other changes. With respect to fluctuating temperature treatments we have recognized for a long time that Q_{10} values are of prime importance. They were included as one of the variables when we calculated coefficients of fluctuation 10 years ago (Powers et al., 1965).

Not only did we observe differences in E_a under some circumstances (Chang and Wu, 1974; Wu et al., 1974), but the optimum temperature for the enzyme shifted when temperature was fluctuated. Furthermore, some of the enzyme units are in a transient (reversible) state (Wu et al., 1975).

If one could wave a magic wand and produce a desired result, that would be great. Enzyme reactions follow some of the same laws as do pure chemical reactions, but unfortunately the conditions affecting the average reaction rates are not as simplistic as Dr. Labuza would have them be.

With reference to Dr. Labuza's remarks concerning Figure 1, a suitable enzyme concentration had to be chosen to give a rate that was slow enough when the temperature was cycled and fast enough to demonstrate possible differences. The values are derived from the results themselves. In the article, we pointed out that both proteolysis and coagulation processes are involved. His assumption based upon enzyme kinetics only is thus faulty.

The authors admit that the statement "this process was not a first order reaction" is ambiguous. We probably should have re-affirmed that more than one process was involved.

As stated in the Introduction, the purpose of this paper was to demonstrate the practical application of FTT. There was a temperature profile for each FTT in our previous paper (Wu et al., 1975). For the rennin study, the temperature change was about 2.5°C/min. The calculation should be:

$$1 = \left[\left(\frac{1}{\theta_{42}}\right)0.5 + \left(\frac{1}{\theta_{40}}\right)0.5 + \left(\frac{1}{\theta_{37}}\right)0.5 + \left(\frac{1}{\theta_{35}}\right)1.5 + \left(\frac{1}{\theta_{37}}\right)0.5 + \left(\frac{1}{\theta_{40}}\right)0.05 + \left(\frac{1}{\theta_{42}}\right)0.5 + \left(\frac{1}{\theta_{45}}\right)1.5\right] \times n$$

(n = number of cycles)

This type of integration has been shown in detail in many papers such as Schwimmer et al. (1955) and Powers et al. (1965). These early studies demonstrated that the integrative effect depends on the magnitude of the Q_{10} value and the amplitude of fluctuation and leads to an increase in the overall "apparent Q_{10}" values.

The second part of the paper demonstrated a more feasible application of FTT. Since the effect of FTT is proportional to the amplitude of the fluctuation and the Q_{10} value of the reaction, and protein denaturation usually is characterized by a high Q_{10} value, a more pronounced effect is apparent if FTT is applied to denaturation processes. Since 10 ml of sample was contained in about 3 ft of 1/8" o.d. coiled steel column and under a pressure of 55 psig, the temperature profile was assumed to be close to square-waved (Schwimmer et al., 1955). The different inactivation rates were integrated during the FTT, thereby taking into account the come-up and come-down times.

The authors admit that the fluctuation of temperature was not fully described in this paper especially for those who have not seen our previous papers.

—*A.C.M. Wu, R.R. Eitenmiller & J.J. Powers,* Dept. of Food Science, Univ. of Georgia College of Agriculture, Athens, GA 30602.

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THE RELATIONSHIP OF CARDIAC SHEAR AND TRACE ELEMENT CONTENT TO BEEF MUSCLE TENDERNESS

ABSTRACT

The purpose of this investigation was to determine whether mechanical shear values of the cardiac muscle could be useful in predicting skeletal muscle tenderness as measured by mechanical shear and sensory evaluation methods. The metal ion content of bovine skeletal muscle was also determined and evaluated for its ability to predict skeletal muscle tenderness. W-B shear results indicate cardiac muscle tenderness is related to semimembranosis tenderness, but the magnitude of the correlation coefficient is not high enough for adequate prediction capabilities. However, the predictive capability of element content is very good for all three tenderness measures–sensory panel, W-B and rotary shear. These results indicate the good potential of this approach to tenderness prediction.

INTRODUCTION

ONE OF THE BASIC difficulties in attempting to predict carcass quality is the inherent variability between various cuts from the same carcass. This presents a very difficult problem in sampling. In an attempt to develop a test method whereby a low cost portion of an animal could be destructively tested to predict the tenderness of more expensive cuts of beef, Anderson (1972) postulated that the heart (cardiac muscle) could possibly be used for this purpose. Results of a preliminary study indicated the potential of this approach. A new mechanical shear apparatus, the rotary shear apparatus, was designed for the purpose of testing this theory (Bjorksten et al., 1967; Anderson, 1972).

The concentration of polyvalent metal ions within the muscle tissue is also seen as being related to meat tenderness. This is based on the fact that nearly all polyvalent metals accumulate on aging, particularly in the aorta, but also in other organs in close contact with blood circulation. Bjorksten et al. (1968) and Bakerman (1969), in describing the crosslinking of collagen theory of aging, listed polyvalent metal ions as being among the main cross-linking agents present within the living system.

The purpose of this investigation was to determine whether mechanical shear values of the heart (cardiac muscle) could be useful in predicting skeletal muscle tenderness as measured by mechanical shear and sensory evaluation methods. Finally, metal ion content of bovine skeletal muscle was determined and also evaluated for its ability to predict skeletal muscle tenderness.

EXPERIMENTAL

Samples tested

Twenty-two bovine hearts, together with the 22 corresponding top rounds from the same carcasses were obtained from a local packing plant. These samples were from 14 USDA choice carcasses, 2 USDA good carcasses, and 6 USDA canner and cutter carcasses.

Two different mechanical shear apparatuses were used for shear analysis, the rotary shear (Anderson, 1972) and the Warner-Bratzler (W-B) shear.

Sampling procedures

The heart samples were obtained directly from the kill floor and analyzed the same day. The auricles and the right ventricle were removed, leaving only the outer wall of the left ventricle and septum for analysis. The cardiac muscle was placed in a polyethylene bag and cooked to an internal temperature of 72°C by immersion in an 80°C constant temperature water bath. Then sample cores (2.54 cm diam) for W-B shear analysis were removed. Rectangular slabs 2 × 3 × 9 cm were removed for rotary shear analysis.

The semimembranosis muscle associated with each heart and carcass was aged in cryovac for 7 days at 5°C prior to analysis. After aging, the muscle was then sectioned into sensory, mechanical shear and chemical portions. The sensory and mechanical shear samples were cooked in a 180°C oven until they reached an internal temperature of 72°C. Then W-B cores and rotary shear slabs were cored and cut to dimensions previously described.

Shear analysis

Shear testing was performed using the W-B shearing device. All W-B shear data were in kg of force (kg-f) required to shear the 2.54 cm core. The rotary shear data were obtained as depth penetrated by the rotating knife after five complete revolutions.

Sensory evaluation

The semimembranosis muscle, the major muscle of the top round was used for sensory evaluation. Samples were cooked as previously described. A nine-member sensory panel was used for evaluation. Panel members were asked to evaluate each sample for tenderness and juiciness on a 5-point hedonic scale ranging from 1.0 (poor) to 5.0 (excellent). Two internal standards (one tough and one tender) were used to normalize the sensory data and thus negate day to day variability in the panel responses.

Trace element analysis

In this investigation, the concentrations of nine different elements within 10 bovine semimembranosis muscles were determined. The 10 muscles were randomly chosen representing each grade. The 10 are made up of 5 choice, 2 good and 3 canner and cutter grades. Seven of the nine elements were analyzed via atomic absorption spectrophotometry (Perkin-Elmer Model 303). These seven were: calcium (Ca), cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), lead (Pb) and zinc (Zn). A

dry ashing technique was applied for the destruction of organic matter (AOAC, 1975).

Selenium (Se) analysis of muscle samples was performed using the colorimetric method described by Cummins et al. (1964, 1965). The method designed for the analysis of silica in water (Rainwater and Thatcher, 1960), was modified for the Si analysis in the muscle samples.

Statistical data analyses

Comparisons of individual data sets with one another were made using the simple correlation analysis as described by Steel and Torrie (1960). Multivarient comparisons of the data sets were accomplished using the stepwise correlation analysis, a statistical method which is a software package of the University of Nebraska Computer Network. All significant tests were performed using the Students' t test at a probability level of 0.05 (Steel and Torrie, 1960).

Table 1—Correlation coefficients obtained upon comparison of cardiac muscle mean shear values versus semimembranosis mean shear values

Shear values compared[a]	Correlation coefficient
W-B (cardiac) vs W-B (semimembranosis)	0.59*
W-B (cardiac) vs Rotary shear (semimembranosis)	−0.60*
Rotary shear (cardiac) vs Rotary shear (semimembranosis)	0.56*
Rotary shear (cardiac) vs W-B (semimembranosis)	−0.55*
W-B (cardiac) vs sensory evaluation (semimembranosis)	0.52*
Rotary shear (cardiac) vs sensory evaluation (semimembranosis)	−0.51*

* Statistically significant at the 0.05 level of probability.
[a] Sample size consists of 22 paired heart samples and corresponding top-round portions.

Table 2—Stepwise multiple linear regression results of element concentration (dry wt basis) vs tenderness as measured by sensory evaluation and shear analysis

Independent variables entered	Dependent variable	Multiple correlation coefficient[a]	Std. error of mean
Fe, Co, Zn, Si	Sensory tenderness	0.97	0.11
Fe, Co, Zn, Ca	W-B shear	0.97	0.62
Fe, Co, Zn, Cu	Rotary shear	0.96	0.07

[a] Sample size consists of 10 semimembranosis muscles

RESULTS & DISCUSSION

Correlation analysis of shear results

Table 1 contains a summary of simple correlation coefficients obtained on comparison of cardiac muscle mean shear values versus semimembranosis muscle mean shear and sensory tenderness values. It can be seen that W-B (cardiac) versus W-B, rotary shear and sensory semimembranosis yielded statistically significant correlation coefficients of 0.59, −0.60 and 0.52, respectively. Similar comparisons were made with rotary shear mean values and yielded similar correlation coefficients of 0.56, −0.55 and 0.51, respectively. Although these results indicate that cardiac muscle tenderness is indeed related to semimembranosis tenderness, the magnitude of the correlation coefficient is not high enough for adequate prediction capabilities.

Trace element analysis results

Of the elements tested, cadmium was found in the lowest amounts in muscle, with concentrations ranging from 0.2–0.4 ppm. Cobalt, selenium, lead and copper were also found in trace amounts, ranging from 1.0 to 4.0 ppm. Zn, Ca, Fe and Si were found in much larger concentrations ranging from 50–200 ppm. Ca was found in the highest concentration, over 200 ppm.

To determine whether a combination of the various element concentrations could be useful in predicting tenderness as measured by shear analyses and sensory evaluation, the data described earlier was subjected to stepwise multiple correlation analysis. In this analysis, the dependent variable (sensory tenderness or shear analysis) was specified by the operator and the computer selected the proper sequence of independent variables (element concentrations) for the linear regression model which most effectively accounted for the variation in the dependent variable. A summary of these stepwise multiple regression results is presented in Table 2. The dependent variables have similar correlation coefficients when four elements are entered, with the sensory panel and the tenderometer having coefficients with a very small error of the mean.

The analysis of sensory tenderness vs element concentrations resulted in a very high multiple correlation coefficient. When the variables Fe, Co, Zn and Si were considered in the linear regression model, a multiple correlation of 0.97 resulted. This corresponded to a standard error of estimation (predic-

Table 3—The ability to predict carcass tenderness as described by sensory score or shear value using the independent variables and the multiple correlation coefficients described in Table 2.

	Actual tenderness measurements			Predicted tenderness from muscle element concentrations		
Carcass no.	Sensory (score)	W-B shear (kg-f)	FSC shear (cm/5 rev)	Sensory (score)	W-B shear (kg-f)	FSC shear (cm/5 rev)
1	4.2	8.3	0.52	3.7	8.7	0.53
2	3.7	10.1	0.64	4.0	10.0	0.70
3	4.3	7.4	0.80	4.3	7.3	0.75
4	4.0	9.0	0.48	4.0	8.5	0.41
5	3.2	10.4	0.57	3.0	9.4	0.58
6	3.1	9.2	0.29	3.3	9.7	0.37
7	3.6	7.6	0.62	3.7	8.5	0.58
8	1.7	16.4	0.22	1.4	16.6	0.18
9	2.2	14.4	0.22	2.3	15.0	0.26
10	1.3	14.8	0.28	1.7	13.7	0.26

tion) of 0.11 and suggests that the regression model has a very good predictive capability.

When W-B shear was compared to element concentration (Fe, Zn, Ca, Co), as shown in Table 2, again there was a high correlation. The very same was found true when the rotary shear was compared to the concentration of the elements Fe, Co, Zn and Cu.

It should be noted that all three multiple correlation runs use three common elements; Fe, Co and Zn.

The ability of the relationship of element content of muscle and its tenderness, as measured by taste panel or shear, to predict tenderness is illustrated in Table 3. The predictive capability is very good for all three tenderness measures: sensory panel, W-B and rotary shear. These results indicate the good potential of this approach to tenderness prediction.

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Ms received 10/18/75; revised 1/16/76; accepted 1/20/76.

Published as Paper No. 4079, Journal Series, Nebraska Agricultural Experiment Station.

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EFFECT OF DEGREE OF VACUUM AND LENGTH OF STORAGE ON THE PHYSICAL CHARACTERISTICS OF VACUUM PACKAGED BEEF WHOLESALE CUTS

ABSTRACT

Wholesale beef cuts were vacuum packaged at low, intermediate or high degrees of vacuum and stored at 1–3°C for 7, 14, 21, 28 or 35 days. Purge loss, trim loss, muscle color and odor were not generally affected by degree of vacuum. Fat appearance and surface discoloration ratings for primal cuts favored the use of a high level of vacuum in three of five storage intervals. Total desirability ratings for primal cuts packaged with high vacuum were higher than those for primal cuts packaged with low vacuum at all five storage intervals. Cuts from the low vacuum treatment had consistently higher numerical values for trim loss than did cuts from intermediate or high vacuum level groups, but these differences were not statistically significant. Degree of vacuum used during storage of primal cuts did not generally affect the peripheral discoloration, surface discoloration or total desirability of subsequent retail steaks. However, among primal cuts stored for 21 days, peripheral discoloration and total desirability scores were lowest ($P < 0.05$) for retail cuts from the low vacuum treatment. Differences in juiciness, flavor desirability, overall satisfaction and Warner-Bratzler shear values for retail steaks were not related to the degree of vacuum used to store primal cuts.

INTRODUCTION

IN 1972, more than half of the fresh beef distributed to retail stores arrived as primal or subprimal cuts; 43% of the subprimals were in vacuum packages and this percentage is expected to reach 65% by 1977 (Shaw, 1973). Cantoni and Bolther (1974) identified the following advantages for vacuum packaging: (a) reduction in weight loss due to dehydration, (b) preservation of muscle color in its freshest state, (c) elimination of external contamination, and (d) prolonged edibility over that of fresh beef. The physical characteristics of greatest concern in vacuum packaged beef are amount of purge and surface discoloration. Purge is meat fluid which exudes from cut surfaces of muscle. In unwrapped cuts, the water evaporates and the protein-myoglobin fraction concentrates on surfaces during prolonged storage. In vacuum packaged beef, the fluid accumulates. Purge is unattractive, supports bacterial growth (thus shortening storage life) and, when allowed to concentrate on the external fat covering, greatly detracts from the freshness and appearance of the wholesale cut. Purge loss approximates 0.1–2% of cut weight and thus is of economic concern.

Urbin and Wilson (1958) evaluated four of the parameters associated with changes in meat color (surface dehydration, temperature, oxygen requirements of the meat surface and bacterial contamination) and reported that vacuum packaging inhibits surface dehydration, reduces the oxygen requirements of meat surfaces and affects bacterial growth. Ledward (1970) claimed that 1–2% oxygen inside the package could theoretically result in the formation of substantial amounts of surface discoloration via metmyoglobin formation.

Two of the most important criteria in describing vacuum packaging systems are the type of film used and the degree of vacuumization achieved. Those films which possess low rates of moisture and oxygen transmission inhibit dehydration of the cut and preclude the re-entry of oxygen after the evacuation of atmospheric air. Johnson (1974) and Ingram (1962) reported that the residual oxygen remaining in a vacuum package after closure is converted to carbon dioxide by respiration of meat tissues and bacteria. Baltzer (1969) reported that carbon dioxide was inhibitory to most of the common aerobic spoilage bacteria.

To prevent dehydration, growth of undesirable aerobic bacteria (e.g., *Pseudomonas* species) and surface discoloration, an impermeable film should be applied in a manner that will completely vacuumize the cut. However, the effect of very high degrees of vacuum on purge loss is not presently known. It is possible that a high degree of vacuum might increase the rate of fluid release from meat, without appreciably affecting the extent of discoloration or bacterial growth. Vacuum packaging studies should include measurements of such traits as purge loss, appearance of primal and subsequent retail cuts, evaluation of organoleptic traits and microbiological growth. Data regarding the microflora of cuts from this study are included in the report of Seideman et al. (1976). The present study determined the effects of degree of vacuum and length of storage on the physical characteristics of vacuum packaged beef wholesale cuts.

EXPERIMENTAL

129 BEEF KNUCKLES (IMPS 167) were prepared, randomly stratified into three equal groups and packaged in a chamber-type vacuum packaging machine at one of three degrees of vacuum. At the time of packaging, these cuts were very fresh, very bright in muscle color, free of surface discoloration and very desirable in general appearance. Cuts in group I were vacuum packaged at the maximum vacuumizing capacity of the machine (chamber vacuum of 29.4 in. of Hg). Cuts in group II were packaged at an intermediate degree of vacuum (chamber vacuum of 28.5 in. of Hg), and cuts in group III were packaged at a low degree of vacuum (chamber vacuum of 26.0 in. of Hg). All cuts were packaged by use of a Conofresh 6000 chamber and Conofresh B (barrier) packaging film. The high degree of vacuum effected complete removal of visible atmospheric air inside the bag, whereas the low degree of vacuum effected partial evacuation of atmospheric air. All of the vacuum packaged cuts were passed through a shrink tunnel (9 sec at an air temperature of 232°C). Primal cuts from each treatment were subjectively scored for degree of vacuum according to a 16-point scale (16 = excellent vacuum, 1 = complete leaker) and randomly assigned to storage intervals of 7, 14, 21, 28 or 35 days. The cuts were boxed and stored for the appropriate storage interval at a temperature of 1–3°C.

Upon completion of each storage interval, individual cuts were weighed and subjectively scored for degree of vacuum. Cuts in packages which received a degree of vacuum score of 1 (leakers) were removed from the study. The remaining (intact) vacuum packages were then opened, the cuts were removed, drained (to remove purge) and weighed. All of the bags were washed, dried and weighed. By the use of differences in weight, a value for percentage of purge loss was obtained. After a bloom period of 1 hr, a two-member highly trained panel sub-

jectively evaluated each cut for fat appearance by use of a 6-point scale (6 = very fresh; 1 = severe or extensive discoloration), for muscle color employing a 9-point scale (9 = very bright cherry red; 1 = black), for surface discoloration by use of a 7-point scale (7 = no surface discoloration; 1 = total surface discoloration), for detectable off-odor employing a 4-point scale (4 = no detectable off-odor; 1 = extreme off-odor), and for total desirability by use of an 8-point scale (8 = extremely desirable; 1 = extremely undesirable). Trim loss percentages were obtained by very carefully removing and weighing areas of discolored lean from the surfaces of cuts.

Following the primal cut evaluation, one retail cut (3 cm in thickness) was removed from each knuckle. Each steak was placed in a plastic foam tray, overwrapped with polyvinyl chloride film and placed under simulated retail display conditions (1–3°C with 90 ft-c of incandescent light). Retail cuts were evaluated at 24 hr intervals for 4 consecutive days by a two-member highly trained panel. Each retail cut was evaluated for surface discoloration by use of a 7-point scale (7 = no surface discoloration; 1 = total surface discoloration), for peripheral discoloration according to a 5-point scale (5 = no peripheral discoloration; 1 = extreme peripheral discoloration) and for total desirability employing an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).

In addition, a second steak (3 cm in thickness) was removed from each primal cut, wrapped, frozen and stored for subsequent taste panel analysis. Steaks were oven-broiled in a 177°C electric oven to an internal temperature of 75°C and presented to an eight-member trained sensory panel. Each steak was evaluated by use of 8-point rating scales for tenderness (8 = extremely tender; 1 = extremely tough), juiciness (8 = extremely juicy; 1 = extremely dry), flavor desirability (8 = like extremely; 1 = dislike extremely) and overall satisfaction (8 = like extremely; 1 = dislike extremely). Furthermore, objective tenderness measurements were made utilizing 1.3 cm cores and a Warner-Bratzler shear machine.

Reduction of data was accomplished using analysis of variance. When significant ($P < 0.05$) main effects were observed in the analysis of variance, mean separation analysis was accomplished using the Kramer (1956) modification of the Duncan (1955) multiple range test.

RESULTS & DISCUSSION

DEGREE OF initial vacuum had no effect on leaker rates. Leaker rates for low, intermediate and high vacuum treatments were 6.5%, 13.6% and 4.5% respectively, following transportation and 2.5%, 5.0% and 2.5%, respectively, following storage. Vacuum scores (Table 1) averaged across storage intervals for low, intermediate and high vacuum treatments were 3.5, 9.8 and 15.4, respectively (initial), and 3.2, 8.9 and 15.1, respectively (terminal). Initial and terminal vacuum scores differed significantly between low, intermediate and high degrees of vacuum at all five storage intervals. Loss of vacuum between initial and terminal evaluations may have resulted from the production of gas inside the bag by heterofermentative bacteria and/or from the passage of atmospheric air through the film. The oxygen transmission rate of the vacuum bag used here was 0.41–0.75 cc/100 sq in./24 hr.

Data in Table 2 suggest that purge losses were not affected by degree of vacuum. Cantoni and Bolther (1974) reported that increased vacuum resulted in greater amounts of purge. Although purge loss percentages (Table 2) generally increased as time in storage increased, the differences were not always consistent enough for statistical significance.

Fat cover appearance scores (Table 3) were significantly higher for high vacuum cuts than for low vacuum samples after 7, 14 and 28 days of storage. This difference probably resulted from greater adhesion of the film to the fat surface on high vacuum samples, thus preventing the absorption of purge fluids by the fat on the surface of such cuts. Fat cover scores were primarily based on the color of fat. Cuts which exhibited discoloration from purge on the fat received inferior scores in comparison to those cuts that were whiter in appearance. With one exception, fat discolored earlier and more extensively on low vacuum samples than on cuts from the high vacuum treatment. Discoloration of subcutaneous fat resulted largely from purge discoloration, but also was associated with increases in bacterial counts (Seideman et al., 1976).

Muscle color (Table 3) was affected by degree of vacuum only at 7 days and increased significantly in brightness on or after 21 days of storage. Production of lactic acid by lactobacilli (which increased greatly between 14 and 21 days of storage, Seideman et al., 1976), may be responsible for the brighter color. Cuts from the low vacuum treatment exhibited significantly greater surface discoloration than did cuts in the high vacuum treatment after 7, 14 and 21 days of storage. After 28 and 35 days of storage, low vacuum cuts had lower mean values for surface discoloration than did the high vacuum samples, but the differences were not consistent enough for statistical significance. Increased surface discoloration of cuts in the low vacuum treatment may have resulted from greater bacterial activity, differences in bacterial types due to availability of oxygen and other gases, or the presence of more residual oxygen which allowed increased formation of metmyoglobin. Discoloration was usually evident in areas where incomplete contact of the muscle surface to the film allowed residual air spaces (Seideman et al., 1976). Surface discoloration did not generally increase as storage time in-

Table 1–Initial and terminal subjective vacuum scores stratified according to degree of vacuum and storage interval

Storage interval (days)	Subjective vacuum score[a]					
	Degree of vacuum					
	Low		Intermediate		High	
	Initial[b]	Terminal[c]	Initial[b]	Terminal[c]	Initial[b]	Terminal[c]
7	3.1[d]	3.1[d]	7.6[d]	6.9[d]	15.4[d]	15.4[d]
14	3.1[d]	2.9[d]	8.1[d]	8.1[d]	15.5[d]	15.3[d]
21	3.9[d]	3.4[d]	10.6[e]	9.3[de]	15.4[d]	14.9[d]
28	3.7[d]	2.9[d]	10.6[e]	8.9[d]	15.3[d]	15.0[d]
35	3.8[d]	3.5[d]	12.0[e]	11.5[e]	15.4[d]	14.9[d]

[a] Means based on a 16-point scale (16 = excellent vacuum; 1 = no vacuum)

[b] Evaluation of package upon arrival. Differences in initial vacuum scores between low, intermediate and high degrees of vacuum were significant at all storage intervals ($P < 0.05$).

[c] Evaluation of package at completion of storage interval. Differences in terminal vacuum scores between low, intermediate and high degrees of vacuum were significant at all storage intervals ($P < 0.05$).

[d,e] Means in the same column bearing different superscripts differ ($P < 0.05$)

Table 2–Purge loss stratified according to degree of vacuum and storage interval

Storage interval (days)	Purge loss (%)		
	Degree of vacuum[a]		
	Low	Intermediate	High
7	0.79[b]	0.99[b]	0.66[b]
14	0.81[b]	0.89[b]	0.79[b]
21	1.09[b]	1.09[b]	1.11[c]
28	1.25[b]	1.78[c]	1.21[c]
35	1.26[b]	1.33[bc]	1.41[c]

[a] No significant differences were observed between degrees of vacuum.

[b,c] Means in the same column bearing different superscripts differ ($P < 0.05$)

Table 3—Appearance of fat cover, muscle color and surface discoloration of primal cuts stratified according to degree of vacuum and storage interval

Storage interval (days)	Fat appearance[a]			Muscle color[b]			Surface discoloration[c]		
	Degree of vacuum[d]			Degree of vacuum[d]			Degree of vacuum[d]		
	Low	Interm	High	Low	Interm	High	Low	Interm	High
7	4.1[e]	4.2[e]	4.7[e]	4.9[e]	5.3[ef]	5.8[ef]	5.3[e]	5.6[e]	6.4[e]
14	4.3[e]	5.0[f]	5.1[f]	5.2[e]	4.9[f]	5.4[e]	5.9[e]	6.3[e]	6.6[e]
21	2.6[f]	3.0[g]	3.0[g]	6.6[f]	5.9[e]	6.4[fg]	5.0[e]	5.5[e]	6.1[e]
28	2.8[f]	3.3[g]	3.9[h]	6.1[f]	5.9[e]	6.5[g]	5.1[e]	5.7[e]	6.0[e]
35	4.0[e]	4.0[e]	4.1[h]	6.3[f]	5.9[e]	6.2[fg]	5.8[e]	5.9[e]	6.1[e]

[a] Means based on a 6-point scale (6 = very fresh; 1 = severe or extensive discoloration)
[b] Means based on a 9-point scale (9 = very light cherry red; 1 = black)
[c] Means based on a 7-point scale (7 = no surface discoloration; 1 = total surface discoloration)
[d] Means within the same storage interval and for the same trait underscored by a common line are not significantly different ($P > 0.05$). There was a significant first-order interaction between degree of vacuum and storage interval for fat appearance ($P < 0.05$).
[e,f,g,h] Means in the same column bearing different superscripts differ ($P < 0.05$).

creased, but was greatest after 21 days for cuts packaged with low or intermediate degrees of vacuum. The latter change coincides with the time of maximum bacterial increase (Seideman et al., 1976). Cuts in the low vacuum treatment decreased slightly, but not significantly, in surface discoloration after 21 days of storage. Watts et al. (1966) reported that reduction of metmyoglobin to myoglobin will not occur until residual oxygen is substantially reduced.

Off-odor is rarely encountered in vacuum packaged beef after short periods of storage because the activity of aerobic spoilage bacteria, primarily pseudomonads, is inhibited. Such was the case in the present study (Seideman et al., 1976). Ingram (1962) reported that aerobic spoilage bacteria, through proteolysis, produce amino compounds that are the principle cause of off-odor. Seideman et al. (1976) reported that the majority (85.7–99.4%) of the bacteria on the cuts after 28 days of storage in the present study were lactobacilli which are facultative anaerobes. Activity of lactic-acid bacteria results in lower meat surface pH, the low pH results in fixation of amino compounds that are produced (Ingram, 1962), and the incidence of off-odor is reduced. With one exception, data in Table 4 reveal no significant differences in off-odor related to either degree of vacuum or storage time.

Total desirability scores were closely associated with ratings for fat cover appearance and surface discoloration. Mean values for total desirability (Table 4) reveal that cuts packaged with low vacuum were significantly less desirable than high vacuum cuts after all five storage intervals. Cuts from low and intermediate vacuum treatments generally decreased in total desirability until 28 days of storage at which time scores tended to improve.

Trim losses are important because they represent a substantial economic loss to retailers. Mean values for trim loss percentage are presented in Table 4. Although mean values were usually highest for the low vacuum cuts and lowest for high vacuum cuts, the differences were not always consistent enough for statistical significance. No significant increases in trim loss were associated with increased storage time among low or intermediate vacuum samples.

Surface discoloration scores for retail steaks are reported in Table 5 and reveal little association with degree of vacuum. Significant decreases in surface discoloration on steaks (from cuts stored 21 and 28 days after 1 day of retail display, from cuts stored 14 days after 2, 3 and 4 days of retail display and from cuts stored 21 and 28 days after 2, 3 and 4 days of retail display) may have resulted from shifts in the microflora on the

Table 4—Odor, total desirability and trim loss for primal cuts stratified according to degree of vacuum and storage interval

Storage interval (days)	Odor[a]			Total desirability[b]			Trim loss (%)		
	Degree of vacuum[c]			Degree of vacuum[c]			Degree of vacuum[c]		
	Low	Interm	High	Low	Interm	High	Low	Interm	High
7	4.0[d]	4.0[d]	4.0[d]	5.8[d]	5.9[de]	6.7[d]	0.01[d]	0.00[d]	0.00[d]
14	4.0[d]	4.0[d]	4.0[d]	5.3[de]	6.6[d]	6.6[d]	0.29[d]	0.03[d]	0.00[d]
21	3.6[e]	4.0[d]	4.0[d]	4.4[e]	5.5[e]	6.6[d]	0.73[d]	0.13[d]	0.00[d]
28	4.0[d]	3.9[d]	3.8[d]	5.2[de]	5.6[e]	6.9[d]	0.43[d]	0.16[d]	0.00[d]
35	3.9[d]	4.0[d]	4.0[d]	5.9[d]	5.9[de]	6.6[d]	0.50[d]	0.36[d]	0.25[e]

[a] Means based on a 4-point scale (4 = no detectable off-odor; 1 = extreme off-odor)
[b] Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable)
[c] Means within the same storage interval and for the same trait underscored by a common line are not significantly different ($P > 0.05$). There were significant first-order interactions between degree of vacuum and storage interval for odor ($P < 0.05$) and total desirability ($P < 0.01$).
[d,e] Means in the same column bearing different superscripts differ ($P < 0.05$).

Table 5—Surface discoloration of retail steaks stratified by day of display, degree of vacuum and storage interval

Storage interval (days)	Surface discoloration[a]											
	Day 1			Day 2			Day 3			Day 4		
	Degree of vacuum[b]			Degree of vacuum[b]			Degree of vacuum[b]			Degree of vacuum[b]		
	Low	Interm	High	Low	Interm	High	Low	Interm	High	Low	Interm	High
7	5.8[c]	5.8[c]	5.9[c]	4.8[c]	4.1[c]	4.6[c]	3.7[c]	3.1[c]	4.0[c]	3.7[c]	3.1[c]	4.0[c]
14	5.9[c]	6.2[cd]	6.1[c]	5.8[d]	6.0[de]	6.0[e]	5.2[d]	5.3[d]	5.0[d]	4.8[d]	4.8[de]	4.5[cd]
21	6.1[c]	6.0[c]	6.2[c]	5.4[d]	5.5[d]	5.5[d]	4.7[d]	5.1[d]	5.3[d]	3.7[c]	4.5[d]	5.1[de]
28	6.9[d]	6.9[e]	6.9[d]	6.3[e]	6.1[e]	6.2[e]	5.9[e]	5.9[d]	6.1[e]	5.5[e]	5.5[de]	5.3[e]
35	6.9[d]	6.8[de]	6.8[d]	6.7[e]	6.5[e]	6.4[e]	6.0[e]	5.9[d]	5.8[e]	5.3[de]	5.4[e]	5.3[e]

[a] Means based on a 7-point scale (7 = no surface discoloration; 1 = total surface discoloration)
[b] Means within the same storage interval and for the same day of display underscored by a common line are not significantly different ($P < 0.05$).
[c,d,e] Means in the same column bearing different superscripts differ ($P < 0.05$)

surfaces of primal cuts, and thus in the types of bacteria which are distributed on retail surfaces during the cutting operation. This shift of the microflora on primal cuts undoubtedly involves a change from bacteria that often cause discoloration of the muscle (e.g., pseudomonads) to bacteria which cause little color damage (e.g., lactobacilli). Seideman et al. (1976) suggested that such a shift actually occurred on samples from the present study.

Mean values for peripheral discoloration of retail steaks are presented in Table 6. Samples from primal cuts that were packaged with a low degree of vacuum exhibited significantly greater peripheral discoloration than did cuts from either the intermediate or high vacuum treatments at the 21-day storage interval. There were no other significant differences in peripheral discoloration attributable to primal cut vacuum treatment.

Mean values for total desirability of retail steaks are presented in Table 7. There were no significant differences in total desirability of retail cuts from knuckles packaged at high, intermediate or low levels of vacuum until the third and fourth days of retail display. Total desirability scores from steaks originating from low vacuum cuts stored for 21 days were significantly lower than were scores for steaks from intermediate and high vacuum treatments after 3 and 4 days of retail display. Total desirability increased as primal cut storage interval increased; scores for steaks were lowest from primal cuts stored 7 days and highest from primal cuts stored 35 days.

Palatability evaluations for steaks from wholesale cuts in each vacuum treatment are presented in Tables 8 and 9. With one exception, degree of vacuum had no significant effect on tenderness evaluated via sensory panel ratings (Table 8) or Warner-Bratzler shear force determinations (Table 9). Tenderness did not increase as storage interval increased, suggesting that increases in tenderness associated with the aging process may have occurred during the first 7 days postmortem. Culp et al. (1973) reported that the aging of carcasses for more than 8–11 days at 1–3°C did little to increase subsequent tenderness of beef.

Degree of vacuum had no significant influence on juiciness ratings (Table 8). Steaks from primal cuts which were packaged with intermediate vacuum and stored for 28 days decreased significantly in juiciness. Neither degree of vacuum nor increasing storage affected flavor desirability of steaks (Table 8). Cantoni and Bolther (1974) reported a maximum tolerable number of 200–300 million lactobacilli per gram to preclude organoleptic changes. This number of lactobacilli was never

Table 6—Peripheral discoloration of retail steaks stratified by day of display, degree of vacuum and storage interval

Storage interval (days)	Peripheral discoloration[a]											
	Day 1			Day 2			Day 3			Day 4		
	Degree of vacuum[b]			Degree of vacuum[b]			Degree of vacuum[b]			Degree of vacuum[b]		
	Low	Interm	High	Low	Interm	High	Low	Interm	High	Low	Interm	High
7	5.0[c]	5.0[c]	5.0[c]	5.0[c]	5.0[c]	5.0[c]	5.0[c]	5.0[c]	5.0[c]	5.0[c]	5.0[c]	5.0[c]
14	4.9[c]	5.0[c]	5.0[c]	4.5[d]	4.7[c]	4.8[c]	4.8[c]	4.4[c]	5.0[c]	4.0[d]	4.3[d]	4.8[c]
21	4.1[d]	5.0[c]	5.0[c]	3.9[e]	5.0[c]	5.0[c]	3.3[d]	4.8[c]	5.0[c]	2.6[e]	4.3[d]	4.9[c]
28	5.0[c]	5.0[c]	5.0[c]	5.0[c]	5.0[c]	5.0[c]	5.0[c]	5.0[c]	5.0[c]	4.9[c]	4.6[cd]	4.9[c]
35	5.0[c]	4.9[c]	4.9[c]	5.0[c]	4.9[c]	4.9[c]	5.0[c]	4.8[c]	4.9[c]	4.8[c]	4.9[c]	4.8[c]

[a] Means based on a 5-point scale (5 = no peripheral discoloration; 1 = extreme peripheral discoloration)
[b] Means within the same storage interval and for the same day of display underscored by a common line are not significantly different ($P > 0.05$). There was a significant first-order interaction between degree of vacuum and storage interval for peripheral discoloration on all 4 days of retail display ($P < 0.001$).
[c,d,e] Means in the same column bearing different superscripts differ ($P < 0.05$).

Table 7—Total desirability of retail steaks stratified by day of display, degree of vacuum and storage interval

Storage interval (days)	Total desirability[a]											
	Day 1			Day 2			Day 3			Day 4		
	Degree of vacuum[b]			Degree of vacuum[b]			Degree of vacuum[b]			Degree of vacuum[b]		
	Low	Interm	High	Low	Interm	High	Low	Interm	High	Low	Interm	High
7	5.4[c]	5.1[c]	5.6[c]	4.9[c]	4.6[c]	5.0[c]	3.6[c]	3.5[c]	3.9[c]	3.6[d]	3.4[c]	3.9[c]
14	6.0[d]	5.9[cd]	5.9[c]	5.8[de]	5.9[de]	5.8[d]	5.6[e]	5.6[d]	5.3[d]	4.5[e]	4.6[de]	4.3[cd]
21	6.2[d]	6.4[de]	6.6[d]	5.5[cd]	5.5[d]	5.8[d]	4.4[d]	5.3[d]	5.3[d]	2.6[c]	4.3[d]	4.9[de]
28	6.9[e]	6.9[e]	6.9[d]	6.5[ef]	6.1[e]	6.2[de]	5.9[e]	5.9[d]	6.1[e]	5.4[f]	5.1[de]	5.3[e]
35	7.2[e]	7.0[e]	6.8[d]	6.8[f]	6.5[e]	6.3[e]	6.1[e]	5.9[d]	5.9[e]	5.3[f]	5.4[e]	5.4[e]

[a] Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable)
[b] Means within the same storage interval and for the same day of display underscored by a common line are not significantly different ($P > 0.05$). There was a significant first-order interaction between degree of vacuum and storage interval for total desirability on the fourth day of retail display ($P < 0.001$).
[c,d,e,f] Means in the same column bearing different superscripts differ ($P < 0.05$).

Table 8—Tenderness, juiciness and flavor desirability of steaks stratified according to degree of vacuum and storage interval

Storage interval (days)	Tenderness[a]			Juiciness[b]			Flavor desirability[c]		
	Degree of vacuum[d]			Degree of vacuum[d]			Degree of vacuum[d]		
	Low	Interm	High	Low	Interm	High	Low	Interm	High
7	5.9[e]	6.2[e]	5.6[e]	5.6[e]	6.2[e]	5.5[ef]	6.1[e]	6.2[e]	5.9[e]
14	5.6[e]	5.6[e]	6.1[e]	5.5[e]	5.7[ef]	5.8[fg]	5.7[e]	5.9[e]	6.1[e]
21	5.7[e]	6.0[e]	6.2[e]	5.9[e]	6.1[e]	6.2[g]	5.6[e]	6.0[e]	6.1[e]
28	5.8[e]	5.5[e]	5.6[e]	5.1[e]	5.0[f]	4.9[e]	5.9[e]	5.6[e]	5.9[e]
35	6.4[e]	5.7[e]	6.4[e]	5.6[e]	5.5[f]	5.6[ef]	6.0[e]	5.8[e]	5.9[e]

[a] Means based on an 8-point scale (8 = extremely tender; 1 = extremely tough)
[b] Means based on an 8-point scale (8 = extremely juicy; 1 = extremely dry)
[c] Means based on an 8-point scale (8 = like extremely; 1 = dislike extremely)
[d] Means within the same storage interval underscored by a common line are not significantly different ($P > 0.05$). There was a significant first-order interaction between degree of vacuum and storage interval for tenderness rating ($P < 0.05$).
[e,f,g] Means in the same column bearing different superscripts differ ($P < 0.05$).

attained in the present study (Seideman et al., 1976) because the storage interval was relatively short and the flavor ratings did not significantly decrease (Table 8).

Overall satisfaction ratings (Table 9) did not differ significantly among cuts packaged with different degrees of vacuum. This was expected since ratings for tenderness, juiciness and flavor desirability were not affected by vacuum level. Overall satisfaction ratings did not vary systematically as a function of storage interval. Overall satisfaction ratings were most closely associated with ratings for juiciness.

CONCLUSIONS

RESULTS of the present study suggest that degree of vacuum had the greatest effect on fat appearance, surface discoloration and total desirability of primal cuts. These differences were the result of failure to completely remove oxygen prior to closure of the vacuum package and from the lack of film to muscle surface contact associated with development of high vacuum. Use of a high degree of vacuum minimizes surface discoloration (by removal of oxygen from the interior of the bag), enhances the appearance of the fat cover (by prevention of purge accumulation on the fat) and improves the general appearance (total desirability) of primal cuts after removal of the packaging material. Degree of vacuum used for storage of

Table 9—Overall satisfaction and Warner-Bratzler shear values of steaks stratified according to degree of vacuum and storage interval

Storage interval (days)	Overall satisfaction[a]			Shear force value[b]		
	Degree of vacuum[c]			Degree of vacuum[c]		
	Low	Interm	High	Low	Interm	High
7	5.9[d]	6.2[d]	5.8[de]	3.7[d]	3.4[d]	4.3[d]
14	5.4[d]	5.7[de]	6.0[d]	3.4[d]	4.3[d]	3.4[d]
21	5.5[d]	5.9[d]	5.9[d]	3.5[d]	3.7[d]	3.5[d]
28	5.7[d]	5.2[e]	5.3[e]	3.3[d]	3.2[d]	3.2[d]
35	5.9[d]	5.7[de]	5.9[d]	3.3[d]	3.4[d]	3.5[d]

[a] Means based on an 8-point scale (8 = like extremely; 1 = dislike extremely)
[b] Average of three determinations per 1.3 cm sample reported in kilograms
[c] Means within the same storage interval and for the same trait underscored by a common line are not significantly different ($P > 0.05$). There was a significant first-order interaction between degree of vacuum and storage interval for overall satisfaction rating ($P < 0.05$).
[d,e] Means in the same column bearing different superscripts differ ($P < 0.05$).

the primal cut had no significant effect on surface discoloration, flavor, juiciness, tenderness or overall satisfaction of retail steaks.

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Ms received 9/12/75; revised 12/13/75; accepted 12/16/75.

T.A. 12134, Texas Agricultural Experiment Station.
This study was supported by the Flexible Packaging Division, Continental Can Company, Inc. and by the USDA Agricultural Marketing Research Institute, ARS, USDA (Contract 12-14-1001-407). This project is a contribution to Western Regional Project WM-62.

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EFFECT OF DEGREE OF VACUUM AND LENGTH OF STORAGE ON THE MICROFLORA OF VACUUM PACKAGED BEEF WHOLESALE CUTS

ABSTRACT

Wholesale cuts of fresh beef were vacuum packaged at low, intermediate or high degrees of vacuum and stored at 1–3°C for 7, 14, 21, 28 or 35 days. Bacterial counts of samples after 7 and 14 days of storage were low [mean count $< 10^4$ per in.^{2} (6.45 cm^2)] irrespective of degree of vacuum. Lactobacilli and anaerobic agar plate counts of cuts stored under high vacuum for 21–35 days tended to be lower than those of comparable cuts stored under low or intermediate vacuum. This was also true, but much less frequently, for the psychrotrophic and mesophilic counts. Largest increases in bacterial counts occurred between 14 and 21 days of storage. Fluorescent pseudomonads represented only a small percentage of the total microbial population of vacuum packaged beef cuts. Lactobacilli and anaerobic plate counts of vacuum-packaged cuts were very similar. The psychrotrophic bacterial population of cuts stored for 28 days consisted primarily of *Lactobacillus* sp., while *Pseudomonas* sp. and *Enterobacteriaceae* represented only a small percentage of the psychrotrophic microflora at that time.

INTRODUCTION

OXYGEN IMPERMEABLE FILMS are effective in extending the shelf-life of fresh meat by controlling the growth of undesirable psychrotrophic bacteria associated with quality deterioration and subsequent spoilage of fresh meat (Baltzer, 1969; Ordal, 1962; Pierson et al., 1970; Johnson, 1974). According to Baltzer (1969), vacuum packaged meat has certain advantages over aerobically packaged meat because: (a) total bacterial counts increase more slowly; (b) putrefaction and slime formation are reduced; and (c) the final bacterial counts after storage usually are lower than in comparable samples packaged in oxygen-permeable films. The principle involved in using oxygen impermeable films is to prevent re-entry of oxygen into the package after residual oxygen is converted to carbon dioxide. Johnson (1974) and Ingram (1962) reported that the oxygen remaining in a vacuum package after closure is converted to carbon dioxide by respiration of meat tissue and bacterial activity. According to Baltzer (1969) and Johnson (1974), the accumulated carbon dioxide inside the package is responsible for the inhibition of pseudomonads, which are often associated with fresh beef spoilage. Johnson (1974) further reported that organisms resistant to carbon dioxide, such as lactic-acid bacteria, can grow slowly at refrigeration temperatures. Jaye et al. (1962) reported that the rate of growth of lactic-acid bacteria on vacuum-packaged meat stored at −1°C was much lower than at 3.3°C.

According to Pierson et al. (1970), vacuum packaging creates an "ecosystem" which is selective with respect to the level and type of microbial flora. Anaerobic conditions suppress the growth of common spoilage bacteria such as *Pseudomonas* and *Achromobacter* species and allow the development of facultative anaerobes such as lactobacilli and *Leuconostoc* species. Jaye et al. (1962), Ingram (1962) and Pierson et al. (1970) reported that lactobacilli become predominant on anaerobically packaged fresh meat.

In recent years, the primary mode of distribution of fresh beef has changed from shipment of carcasses to that of vacuum packaged primal and subprimal cuts. Defects in organoleptic and appearance traits of vacuum packaged beef cuts caused by microbial activities have been reported. Such defects include off-flavors, off-odors and undesirable colors after 8–11 wk of refrigerated storage (Johnson, 1974). Pierson et al. (1970) attributed the sour flavor of vacuum packaged beef to the development of lactic-acid bacteria.

Most of the previous research in this area has dealt with vacuum packaged processed meat or Saran-wrapped ground beef. There has been little research on the microflora of wholesale cuts of vacuum packaged beef. This study reports on the microflora of vacuum packaged fresh beef cuts over a 35-day storage period and on the effect of three degrees of vacuum on the microflora of wholesale beef cuts. Data regarding the physical characteristics of these and additional cuts are included in the report by Seideman et al. (1976).

EXPERIMENTAL

69 BEEF KNUCKLES (IMPS 167) were randomly divided into three groups and were vacuum packaged in a commercial packing plant at three different degrees of vacuum. A chamber-type vacuum packaging machine (Conofresh 6000) was used with film bags with the following characteristics: oxygen transmission rate of 0.41–0.75 cc/100 in.^{2}/24 hr/75°F/50% RH; moisture vapor transmission rate of 0.18–0.20 g/100 in.^{2}/24 hr/100°F/70% RH. Group 1 was vacuum packaged at maximum capacity of the vacuum packaging machine, with a chamber vacuum of 29.4 in. of Hg. Group 2 was packaged at an intermediate degree of vacuum (chamber vacuum of 28.5 in. of Hg) and Group 3 was packaged at a low degree of vacuum with a chamber vacuum of 26.0 in. of Hg. The use of a low degree of vacuum resulted in only partial evacuation of atmospheric air. The reported chamber vacuum should not be considered as the actual vacuum inside the bag. Immediately after packaging, all cuts were passed through a shrink tunnel and were exposed to an air temperature of 232°C for 9 sec. Packaging films then were removed from three randomly selected packages from each treatment to obtain samples for initial bacterial counts. The remaining 20 cuts from each treatment were randomly assigned to storage periods of 7, 14, 21, 28 or 35 days at 1–3°C (four cuts per storage interval).

An objective vacuum measurement was made on each package to determine the actual vacuum inside the package. The measurement was performed by submerging each package under water in a glass desiccator jar. A vacuum was created within the desiccator jar by the use of an electric vacuum pump. When the film separated from the meat surface a measurement in inches of Hg was recorded.

At the end of each storage period, leaker packages, as determined subjectively, were removed from the study and the remaining intact packages were opened.

Bacteriological evaluation of the cuts was performed by sampling the surfaces of the cut with a damp, sterile 5 × 5 × 1.3 cm cellulose sponge, with the analyst's hand covered with a sterile, plastic glove (Silliker and Gabis, 1975). Before sampling, the sponge was wetted in sterile 0.1% peptone broth (100 ml in glass canning jars) and the excess broth was removed by squeezing. An area 12.7 cm long and 5 cm wide was swabbed, the sponge was then inverted and an additional area (12.7 cm long) was swabbed (total area of 129 cm^2). Each cut was sampled in

the same manner, first the sirloin face (64.5 cm^2) and then the subcutaneous fat cover (64.5 cm^2). The sponge was then placed in the 100 ml sterile 0.1% peptone broth and squeezed in this broth five times. The sampling jar was shaken 25 times and appropriate dilutions were made with sterile 0.1% peptone broth.

A psychrotrophic bacterial count was obtained by the spread plate technique by placing 0.1 ml of appropriate dilutions on plate count agar (Difco). The inoculum was spread evenly over the surface of the agar plate with a sterile bent glass rod. Plates were incubated for 10 days at 7°C. A mesophilic bacterial count was obtained by the spread plate technique utilizing plate count agar with subsequent incubation of plates at 32°C for 3 days. Pseudomonas agar F (Difco) was used to obtain a count of fluorescent pseudomonads using the spread plate technique with plate incubation for 2 days at 25°C. Enumeration of lactobacilli was carried out by the pour plate technique utilizing Lactobacillus MRS broth (Difco) with 1.5% agar added. Plates were incubated for 5 days at 32°C. An anaerobic plate count was obtained on trypticase soy agar (BBL) with the pour plate technique and incubation was for 5 days at 32°C in anaerobic jars (GasPak, BBL). To determine the distribution of the microflora of refrigerated vacuum packaged beef cuts, 30–40 colonies were picked at the 28-day storage interval from countable plates used for the psychrotrophic bacterial counts. Diagnostic schemes and procedures for identification of isolates were those presented by Vanderzant and Nickelson (1969).

Retail cuts (about 3 cm thick) were removed from primal cuts, placed in plastic foam trays and overwrapped with polyvinyl chloride film. The retail cuts were placed under simulated retail display conditions at 1–3°C with 90 ft-c of incandescent light. Bacterial counts were obtained from steaks after 4 days of retail display by swabbing 12.9 cm^2 with sterile cotton swabs. This technique was used due to the limited surface area available for sampling.

Data in the tables represent the mean of four meat cuts. Data were analyzed by analysis of variance on log counts of individual samples. When mean (geometric) counts were different ($P < 0.05$), the technique of Duncan (1955) was employed for mean separation.

RESULTS

OBJECTIVE terminal vacuum measurements (Table 1) were obtained as a more accurate measure of vacuity inside the package than that provided by use of the degree of vacuum in the chamber at the time of packaging. The packages stored under high vacuum did not have visible residual air spaces between the meat surface and the film, with the film adhering tightly to the meat surface. Samples in the low vacuum group had numerous visible air spaces between the meat surface and film. Mean values for terminal vacuum measurements reveal significant differences among levels of vacuum used for packaging. Objective vacuum measurements averaged over storage intervals for cuts in low, intermediate and high vacuum treatments were 11.2, 16.6 and 25.8 in. of Hg, respectively.

Psychrotrophic bacterial counts of samples stored for 0–35 days at 1–3°C under three different degrees of vacuum are presented in Table 2. Psychrotrophic counts initially and after 7 and 14 days at 1–3°C were low (less than 10,000/$in.^2$). After 7, 14 and 21 days, psychrotrophic counts of cuts stored under high vacuum were numerically lower than those of cuts stored under low vacuum. Significant differences ($P < 0.05$) in psychrotrophic count because of extreme differences in degree of vacuum (low vs high) were observed after 14 and 21 days of storage. The largest increases in psychrotrophic counts (2.14–2.5 logs) occurred between 14 and 21 days of storage, irrespective of vacuum treatment. Psychrotrophic counts of approximately 10^6 per $in.^2$ (6.45 cm^2) were reached first in the samples stored for 21 days under low vacuum and then in all samples at 28 days. Increases in psychrotrophic counts of cuts stored from 21–28 days and from 28–35 days were small (0.38–1.15 and 0.47–0.72 logs) compared to those observed between 14 and 21 days of storage.

Mesophilic bacterial counts of cuts stored for 0–35 days at 1–3°C under different degrees of vacuum are presented in Table 3. The pattern of changes in mesophilic bacterial counts was similar to that described for psychrotrophic bacterial counts (Table 2). Mesophilic counts of cuts initially and after

Table 1–Terminal, objective measurements of vacuum for beef knuckles stratified according to degree of vacuum and storage interval

Storage interval (days)	Objective vacuum measurements[a]		
	Degree of vacuum		
	Low	Intermediate	High
7	11.0[b]	19.5[b]	25.6[c]
14	15.8[b]	17.5[b]	28.0[b]
21	11.0[b]	18.0[b]	22.5[d]
28	7.5[b]	11.7[b]	26.3[c]
35	10.5[b]	16.5[b]	26.5[c]

[a] Objective measurements, reported in inches of mercury (Hg), taken at the termination of each storage interval. Means on the same row underscored by a common line are not significantly different ($P > 0.05$).

[b,c,d] Means ir the same column bearing different superscripts differ ($P < 0.05$).

Table 2–Psychrotrophic bacterial counts of beef knuckles stratified according to degree of vacuum and storage interval

Storage interval (days)	Psychrotrophic bacterial count[a]		
	Degree of vacuum[b]		
	Low	Intermediate	High
0	1.50[c]	1.90[c]	1.40[c]
7	3.06[d]	1.47[c]	2.01[d]
14	3.49[d]	3.52[d]	2.78[e]
21	5.99[e]	5.66[e]	5.27[f]
28	6.37[ef]	6.39[f]	6.42[g]
35	7.05[f]	7.11[g]	6.89[g]

[a] Counts ($\log_{10}$) per $in.^2$ (6.45 cm^2)

[b] Means within the same storage interval underscored by a common line are not significantly different ($P > 0.05$). There was a significant first-order interaction between degree of vacuum and storage interval for psychrotrophic bacterial count ($P < 0.05$).

[c,d,e,f,g] Means in the same column bearing different superscripts differ ($P < 0.05$).

Table 3–Mesophilic bacterial counts of beef knuckles stratified according to degree of vacuum and storage interval

Storage interval (days)	Mesophilic bacterial count[a]		
	Degree of vacuum[b]		
	Low	Intermediate	High
0	1.79[c]	1.49[c]	1.96[c]
7	4.76[d]	1.59[c]	2.36[cd]
14	3.26[e]	3.50[d]	3.35[d]
21	5.83[f]	5.51[e]	5.37[e]
28	6.40[fg]	6.17[f]	6.61[f]
35	7.05[g]	7.06[g]	6.82[f]

[a] Counts ($\log_{10}$) per $in.^2$ (6.45 cm^2)

[b] Means within the same storage interval underscored by a common line are not significantly different ($P > 0.05$). There was a significant first-order interaction between degree of vacuum and storage interval for mesophilic bacterial count ($P < 0.001$).

[c,d,e,f,g] Means in the same column bearing different superscripts differ ($P < 0.05$).

7 and 14 days at 1–3°C were low. Mesophilic counts after 21, 28 and 35 days were similar to the psychrotrophic counts of comparable cuts. The largest increases in count (2.01–2.57 logs) occurred between 14 and 21 days of storage. Although mesophilic counts between cuts packaged with different degrees of vacuum did not differ significantly ($P > 0.05$) mean counts of cuts stored for 21 and 35 days under high vacuum were numerically lower than were those of cuts stored under low or intermediate vacuum.

Fluorescent pesudomonad counts are presented in Table 4. Fluorescent colonies, when detected, usually were on crowded plates of lower dilutions and were frequently difficult to count. The data, however, show that fluorescent pseudomonads constituted only a small fraction (mean count $\leq 10^4$ per in.^{2}) of the microflora which developed on vacuum packaged beef cuts.

Changes in the level of lactobacilli on vacuum packaged beef cuts during refrigerated storage are presented in Table 5. In preliminary experiments, biochemical tests indicated that the majority of the colonies developing on the lactobacilli agar were *Lactobacillus* species. After 21–35 days, lactobacilli counts of samples stored under high vacuum were numerically lower than those stored under intermediate or low vacuum. Statistically significant differences in lactobacilli counts because of differences in vacuum were noted only after 7 and 21 days of storage. Lactobacilli counts initially and after 7 and 14 days of storage were low. The largest increases in count (2.28–3.09 logs) were observed between 14 and 21 days of storage.

Anaerobic plate counts of beef cuts stored for 0–35 days at 1–3°C are presented in Table 6. No statistically significant differences in counts could be attributed to degree of vacuum but numerical counts of cuts stored for 21, 28 and 35 days under high vacuum tended to be lower than those of comparable cuts stored under intermediate or low vacuum. The largest increases in anaerobic counts (2.4–3.1 logs) occurred between 14 and 21 days of storage.

The percentage distribution of microbial types (Table 7) shows that *Lactobacillus* sp. dominated the microbial population of the samples stored for 28 days, irrespective of degree of vacuum. The highest percentages of lactobacilli were found on cuts stored under high vacuum. *Pseudomonas* sp. and *Enterobacteriaceae* represented only a small fraction of the total population after 28 days of storage and were usually less

Table 4—Fluorescent pseudomonad counts of beef knuckles stratified according to degree of vacuum and storage interval

Storage interval (days)	Fluorescent pseudomonad count[a]		
	Degree of vacuum[b]		
	Low	Intermediate	High
0	1.39[c]	1.39[c]	1.39[c]
7	1.59[c]	1.39[c]	1.39[c]
14	2.23[c]	2.32[cd]	1.79[c]
21	3.18[c]	2.44[cd]	1.93[c]
28	3.39[c]	3.80[d]	4.01[d]
35	3.04[c]	2.69[cd]	2.54[cd]

[a] Counts ($\log_{10}$) per in.^{2} (6.45 cm^2)
[b] Means within the same storage interval underscored by a common line are not significantly different ($P > 0.05$).
[c,d] Means in the same column bearing different superscripts differ ($P < 0.05$).

Table 5—Lactobacillus agar counts of beef knuckles stratified according to degree of vacuum and storage interval

Storage interval (days)	Lactobacillus agar count[a]		
	Degree of vacuum[b]		
	Low	Intermediate	High
0	1.76[c]	1.40[c]	1.83[c]
7	2.96[c]	1.40[c]	1.70[c]
14	2.66[d]	2.68[d]	2.80[d]
21	5.75[e]	5.41[e]	5.08[e]
28	6.26[ef]	6.13[f]	6.01[f]
35	6.78[f]	6.93[g]	6.62[g]

[a] Counts ($\log_{10}$) per in.^{2} (6.45 cm^2)
[b] Means within the same storage interval underscored by a common line are not significantly different ($P > 0.05$).
[c,d,e,f,g] Means in the same column bearing different superscripts differ ($P < 0.05$).

Table 6—Anaerobic plate counts of beef knuckles stratified according to degree of vacuum and storage interval

Storage interval (days)	Anaerobic plate count[a]		
	Degree of vacuum[b]		
	Low	Intermediate	High
0	1.49[c]	1.39[c]	1.39[c]
7	1.79[c]	1.98[c]	1.82[c]
14	2.67[d]	3.05[d]	2.77[d]
21	5.77[e]	5.42[e]	5.17[e]
28	6.33[f]	6.23[f]	6.11[f]
35	6.96[g]	6.87[g]	6.68[f]

[a] Counts ($\log_{10}$) per in.^{2} (6.45 cm^2)
[b] Means within the same storage interval underscored by a common line are not significantly different ($P > 0.05$).
[c,d,e,f,g] Means in the same column bearing different superscripts differ ($P < 0.05$).

Table 7—Percentage distribution of microorganisms on beef knuckles stored at 1–3°C for 28 days stratified according to degree of vacuum

Microbial type	Percentage distribution of microbial flora[a]					
	Degree of vacuum[b]					
	Low		Intermediate		High	
	Min	Max	Min	Max	Min	Max
Lactobacillus sp.	88.4	95.8	85.7	96.7	98.0	99.4
Pseudomonas sp.	0	8.6	0	3.3	0	0.2
Enterobacteriaceae	0	4.7	0	12.8	0.4	2.4
Yeast	0	4.6	0	0	0	0

[a] Each percentage is based on three to four analyses.
[b] No statistical analyses were performed on these data.

numerous on cuts stored under high vacuum than on comparable cuts stored under low or intermediate vacuum.

Psychrotrophic bacterial counts of steaks from cuts stored under different conditions of vacuum for various storage times are presented in Table 8. Counts of approximately 10^6 per in.^{2} were reached first in steaks from cuts stored for 28 days at either low or intermediate vacuum. Retail steaks from cuts stored for 35 days at the three vacuum levels had counts exceeding 10^6 per in.^{2}. Psychrotrophic counts of steaks prepared from cuts stored for 28 days under high vacuum were 0.65–1.07 log lower than those from cuts stored under low or intermediate vacuum. The differences in psychrotrophic counts of the steaks due to variation in degree of vacuum of the primal cuts were not statistically significant.

DISCUSSION

WITHIN THE LIMITS of this study (particularly with respect to type of packaging film, level of vacuum, storage temperature and duration of storage) vacuum packaging of fresh beef primal cuts under high vacuum was slightly superior microbiologically to packaging at low or intermediate degrees of vacuum. Bacterial counts initially and after 7 and 14 days were low. Destruction and/or sublethal injury of bacteria on the surface of the beef by heat during passage through the shrink tunnel may have been a contributing factor.

The largest increases in psychrotrophs, mesophiles, lactobacilli and anaerobes occurred consistently between 14 and 21 days of storage, irrespective of degree of vacuum. Although the exact reasons for this are not known, it is possible that the gaseous environment inside the packages during this period was optimum for the development of lactic-acid bacteria. In addition, biochemical activities (proteolysis, for example) by other microbial species during the first 14 days may have provided a more desirable microenvironment for lactic acid bacteria. Furthermore, some time may also be required for the recovery of sublethally injured bacteria.

After 21 days, lactobacilli and anaerobic plate counts of cuts stored under high vacuum tended to be lower than those of comparable cuts stored at either low or intermediate vacuum. A similar pattern, but occurring much less frequently, was noted for the psychrotrophic and mesophilic bacterial counts. Differences in the level and growth rate at 1–3°C of lactic acid bacteria and gram-negative bacteria such as *Pseudomonas* and *Enterobacteriaceae* may explain this. At the lower vacuum levels, *Pseudomonas* and *Enterobacteriaceae* often were more numerous than at the high vacuum level (Table 7). A somewhat higher growth rate of the gram-negative species at 1–3°C as compared to that of the lactic acid bacteria may be partially responsible for these differences in count. Although the composition of the gases in the packages were not determined, it is likely that differences in gaseous environment will influence the percentage distribution of microbial species both through inhibitory and stimulating actions.

After 35 days of storage at 1–3°C the mean psychrotrophic count of cuts stored under high vacuum was 7.8×10^6, those for cuts stored under low and intermediate vacuum were $11–13 \times 10^6$ per in.^{2}; comparable figures for lactobacilli were 4.2×10^6 vs $5.0–8.5 \times 10^6$ per in.^{2}. In general, psychrotrophic and mesophilic counts after 21–35 days were similar for cuts stored under identical conditions of vacuum. This indicates that the principal bacteria on the beef cuts were capable of growth over a wide range of temperatures. This is a common characteristic of psychrotrophic bacteria of fresh beef. Counts for lactobacilli and anaerobes of cuts stored under comparable conditions also were similar indicating that the predominant microbial types were capable of growth with and without oxygen. In this respect, Jaye et al. (1962) and Pierson et al. (1970) demonstrated that lactic acid bacteria developed about equally well at 3.3°C in aerobically packaged and anaerobically packaged beef. On the other hand, counts for lactobacilli or anaerobes of cuts stored for 35 days were considerably lower than psychrotrophic or mesophilic counts of comparable cuts stored under identical conditions. Differences in composition of the plating media (plate count agar, MRS broth with agar added, trypticase soy agar) and in conditions of plate incubation (10 days at 7°C or 3 days at 32°C vs 5 days at 32°C) are most likely responsible for these differences in counts.

Counts for psychrotrophs, mesophiles and lactobacilli of vacuum packaged beef ($10^{6.6}–10^{7.1}$ per in.^{2}) after 35 days at 1–3°C are in general agreement with those reported by Johnson (1974). Johnson (1974) reported that properly processed vacuum packaged beef stored at 0°C should keep for about 11 wk and that bacteria reach maximum numbers of the order of 10^7 per cm^2 during the fifth week of storage. That vacuum-packaging reduced the aerobic plate count (both psychrotrophic and mesophilic) of beef cuts was obvious when comparing intact packages and leakers. Counts of leaker packages (after 21–35 days) were often 2–4 logs higher than those from intact packages.

Fluorescent pseudomonads constituted only a small fraction of the microbial flora of vacuum packaged beef. Small increases in fluorescent pseudomonads (1.2–1.7 logs) occurred over a 35-day storage period with mean counts after 35 days ranging from $10^2–10^3$ per in.^{2}. Jaye et al. (1962), Ordal (1962) and Pierson et al. (1970) reported that vacuum packaging of meat repressed the development of fluorescent pseudomonads.

Lactobacillus sp. were predominant on vacuum packaged beef stored for 28 days. Beebe (1975) reported that gram-negative rods, primarily *Pseudomonas* sp., were predominant on leaker packages. Similar results have been reported by Pierson et al. (1970) who reported that lactic acid bacteria accounted for about 90% of the total bacterial flora of anaerobically packaged beef stored for 15 days at 3.3°C. According to Jaye et al. (1962), lactobacilli became predominant in anaerobically packaged ground beef after storage for 8 days at 3.3°C. The somewhat lower levels of *Pseudomonas* and *Enterobacteriaceae* on cuts stored under high vacuum might be considered an advantage of this treatment. Arafa and Chen (1975) reported that 95.9% of the microorganisms on vacuum packaged poultry at the point of spoilage were *Enterobacter* sp. and that members of the *Enterobacteriaceae* grew in vacuum packaged fresh poultry. Since some members of the latter family are well-known pathogens involved in foodborne illness, the po-

Table 8—Psychrotrophic bacterial counts of retail steaks after 4 days of display (1–3°C) stratified according to degree of vacuum and storage interval

Storage interval (days)	Psychrotrophic bacterial count[a]		
	Degree of vacuum[b]		
	Low	Intermediate	High
7	2.71[c]	3.02[c]	2.77[c]
14	2.60[c]	2.27[c]	2.70[c]
28	5.96[d]	6.38[d]	5.31[d]
35	6.22[d]	6.36[d]	6.30[d]

[a] Counts ($\log_{10}$) per in.^{2} (6.45 cm^2)
[b] Means within the same storage interval underscored by a common line are not significantly different ($P > 0.05$).
[c,d] Means in the same column bearing different superscripts differ ($P < 0.05$).

tential growth of *Enterobacteriaceae* in vacuum packaged meats warrants further study.

This report and others (Jaye et al., 1962; Pierson et al., 1970; Johnson, 1974) show that lactic-acid bacteria become predominant in refrigerated vacuum packaged beef. Defects (sour or cheesy flavors and off-colors) caused by microbial activities in vacuum packaged beef cuts have been observed (Johnson, 1974). The results of this study were used to explain variations in certain physical characteristics of vacuum-packaged beef wholesale cuts and organoleptic ratings of steaks from these cuts (Seideman et al., 1976). There was possible bacterial involvement in: (a) less acceptable appearance of the fat cover between 14 and 21 days; (b) brighter muscle color after 21 days; (c) increased surface discoloration on cuts in low vacuum treatments; and (d) increased incidence of off-odor for cuts stored at a low degree of vacuum for 14–21 days. The microbiological study also supports the conclusion that the flavor of steaks was not affected by degree of vacuum or length of storage of the vacuum packaged wholesale cuts (Seideman et al., 1976). To maintain adequate shelf life it is necessary: (a) to package beef with a low initial bacterial count and (b) to maintain low storage temperatures (−1 to 0°C) (Johnson, 1974). The first objective can be achieved by rigorous sanitary procedures and plant sanitation. The importance of low storage temperatures on controlling the development of lactic-acid bacteria was demonstrated by Jaye et al. (1962). Jaye et al. (1962) reported that lactic acid bacteria grew well in ground beef at 3.3°C both in aerobically and anaerobically packaged beef but were suppressed greatly at −1°C.

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Ms received 9/12/75; revised 12/13/75; accepted 12/16/75.

T.A. 12133, Texas Agricultural Experiment Station.

This research was supported by the Flexible Packaging Division, Continental Can Company, Inc. and the USDA Agricultural Marketing Research Institute, ARS, USDA (Contract 12-14-1001-407). This project is a contribution to Western Regional Project WM-62.

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EFFECT OF PROCESSING CONDITIONS AND ADDITIVES ON THE TEXTURE OF STORED FREEZE-DRIED BEEF

ABSTRACT

The effect of processing variables on the texture of cooked, freeze-dried, beef top round has been studied. Treatments included "tenderstretch" hanging, aging, cooking temperature and time, infusion with salt solutions and use of antioxidants. Texture of meat was improved by "tenderstretch" hanging and rehydration in papain, while there was a large increase in texturometer hardness on high temperature storage. Increasing cooking temperatures resulted in increased hardness up to 75°C, but at 85°C and above decreases occurred. Cooking for 4 hr as compared to 2 hr had no effect at cooking temperatures below 75°C but at 85°C caused a decrease in hardness. Cooking temperature and time did not affect the storage properties of freeze-dried beef, but corning, infusion with sodium and potassium chlorides and aging all reduced the hardness increase caused by high temperature storage.

INTRODUCTION

TEXTURE, particularly after storage, is one of the major problems associated with freeze-dried meat and a variety of factors has been found to affect the textural quality.

Aging improves the tenderness of meat by breakdown in the Z-line region of the myofibrils (Davey and Gilbert, 1969) decreasing their strength and hence the shear strength. It would be expected that improvements in tenderness brought about by aging would be carried over to the freeze-dried product.

"Tenderstretch" method of hanging the carcass, increases sarcomere length in many muscles of the hindquarter resulting in improved tenderness (Hostetler et al., 1972). As the increased sarcomere length will be unaffected by freeze drying it is expected that freeze-dried "tenderstretch" meat will be more tender than that hung conventionally.

The effect of cooking temperature and time on the quality of freeze-dried beef is not clear. Tuomy et al. (1968) found that higher internal temperatures attained during cooking resulted in higher taste panel scores for flavor, odor and texture. Hinnergardt (1974) has indicated that beef roasted to 60°C internal temperature and stored at 38°C for 6 months following freeze drying had texture and color more acceptable to taste panel than beef roasted to 71°C.

Hamdy et al. (1959) improved the water-holding capacity (WHC) of fresh and freeze-dried beef by incorporation of sodium and potassium chlorides and ascorbic acid into the meat before cooking. Improvement of WHC was also noted when freeze-dried beef was rehydrated in solutions of these salts.

Pyrophosphate is often used with meats because of its ability to increase WHC, partly due to increased pH (Shults et al., 1972). The pH of meat is known to have large effects on WHC, shear, hardness, and subjective tenderness (Penny et al., 1963; Bouton et al., 1973).

Autoxidizing lipids are associated with crosslinking of proteins (Andrews et al., 1965) and thus possibly lead to toughening of stored, freeze-dried meat. Antioxidants were found to prevent the development of rancid odors and flavors in a variety of freeze-dried meats (Fairbrother and Younger, 1967), but the effect on tenderness has not been studied.

The effects of processing variables on hardness and the increase in hardness associated with high temperature storage of freeze-dried beef top round are reported here. Variables studied include hanging method, aging, cooking temperature and time, and the addition of various salts before cooking or during rehydration.

MATERIALS & METHODS

Meat

Beef top round were obtained from the local slaughter house as required or frozen, "export quality," top round were used. Meat obtained locally was cooked 2–5 days postmortem.

Freeze drying and storage

Cooked meat was sliced, in general, to a 10 mm thickness, unless otherwise mentioned, using an electric meat slicer and frozen at −18°C in still air. Freeze drying was carried out in a modified Vickers Pilot Accelerated Freeze Drying (AFD) plant using contact heating, and a plate temperature of 50°C and chamber pressure of less than 13 Pa unless otherwise stated.

Freeze-dried samples were vacuum packed in laminate pouches (polyester/polyethylene/foil/polyethylene) at less than 7 kPa and stored at 1 and 37°C for 90 days before rehydration.

Rehydration

Unless stated otherwise in the text, freeze-dried samples were rehydrated by placing in cold water or other stated solution for 5 min, when they were turned over and the solution heated to ca 85°C on a hot plate to give a total rehydration time of 15 min. Samples were tested by texturometer within 10 min of completing rehydration.

Texturometer testing

Hardness was measured on a Zenken Texturometer (Tokyo) and is expressed as force, in Newtons, to compress a 10 mm slice to 2 mm with an 18 mm diameter plunger.

Taste panel testing

Samples were submitted to a taste panel of five panelists, experienced in meat tasting, for assessment of juiciness, tenderness and overall texture using 9-point hedonic rating scales, "9" being extremely juicy or tender, or like extremely and "1" being extremely dry or tough, or dislike extremely, and neutral point of "5."

Hanging and aging

Four steers were slaughtered locally and after splitting, one-half of the carcass was hung conventionally by the Achilles tendon, the other half by the obturator foramen ("tenderstretch" method) till boning out. Muscle semimembranosus from each side was cut in half, one-half being cooked 4–5 days postmortem, and the other stored at 4°C for a further 14 days before cooking. Cooking was carried out to an internal temperature of 75°C in an atmospheric steam cooker, taking 1–2 hr depending on shape and weight. The cooled, sliced meat was freeze dried and after storage, rehydrated as described earlier.

Internal temperature attained during cooking

Eight top rounds from four steers were each cut in half and the four

pieces from each steer randomized among the cooking treatments. Cooking was carried out in an atmospheric steamer, meat being removed when internal temperatures of 50, 75, 85 and 98°C were reached. These internal temperatures were attained after 2¼–3, 3½–5½, 3¾–5½ and 4½–6 hr respectively, time depending on size and shape of the meat sample.

The freeze-dried meat was vacuum packed at less than 7 kPa and stored, rehydrated and tested as described.

Cooking temperature and time

The two M. semimembranosus from four steers were cut into four pieces of 0.4–0.8 kg and the eight pieces per steer randomized among four cooking temperatures, 55, 65, 75 and 85°C, for two cooking times of 2 and 4 hr. Samples were vacuum packed in polyethylene bags and cooked in a water bath maintained at the desired temperature, internal temperatures reaching that of the bath in 1¾–2 hr.

Samples were freeze dried, stored and tested as described.

Infusion with salt solutions

Four M. semimembranosus were each cut into five pieces, and these pieces were injected with the following solutions to 10% of raw weight:

A–control, no treatment;
B–7% sodium chloride, 1.2% potassium chloride and 0.02% ascorbic acid;
C–7% sodium chloride and 1.2% potassium chloride;
D–0.02% ascorbic acid;
E–14% sodium chloride, 6% sucrose, 0.6% sodium nitrate and 0.1% sodium nitrite.

Injected meat samples were stored overnight at 1°C before cooking in atmospheric steam to 75°C internal temperature. Drip loss was determined by weighing the meat, raw and after cooking, cooling and removal of excess water by blotting with paper towelling.

Slices were freeze dried and storage, rehydration and "texturometer" testing carried out as described.

Corning components

Four beef semimembranosus were cut into five pieces of 0.2–0.5 kg, and randomized among the five experimental treatments of untreated control or injection to 10% of raw weight with solutions containing one of 60g sucrose, 140g sodium chloride, 6g potassium nitrate or 1g sodium nitrite per liter of water.

After standing overnight at 1°C, samples were sealed in polyethylene bags and cooked by immersion in water at 75°C for 2 hr. Previous results indicate that the center temperatures of meat samples of this size approach the bath temperature in 1½–2 hr.

Samples were freeze dried as previously described, vacuum packed and stored for 90 days at 1 and 37°C before rehydration and testing by texturometer.

Evaporative freezing vs pre-freezing and rehydration with papain, pyrophosphate and citrate solutions

The two M. semimembranosus from each of four steers were cooked to 75°C internal temperature in an atmospheric steam cooker, time taken being 1¼–2 hr. After cooking, and slicing to 10 mm thickness, slices from one side of each steer were frozen at −18°C in still air and freeze dried as previously described. The cooked slices from the other side were frozen by evaporative cooling in the freeze drier, sample internal temperature dropping to −20°C within 10 min of starting the vacuum pump, and taking less than 2 min to pass through the freezing zone. Freeze drying was then carried out as before. Packaging and storage were as described.

Samples were rehydrated as previously described in solutions containing 0, 5, 20 and 100 ppm papain (BDH) and 0, 0.5 or 1.0% sodium citrate or 0.5% tetra-sodium pyrophosphate decahydrate before testing.

M. adductor from the four steers were cooked to 75°C internal temperature and after slicing to 10 mm were frozen at −18°C in still air and freeze dried as for the semimembranosus. After storage for 90 days at 1 and 37°C, samples were randomized among the 16 rehydrating solutions and subjected to a taste panel.

Antioxidants

The M. semimembranosus from four, beef top rounds were cooked to 75°C internal temperature in a steam cooker over 2–2½ hr. After slicing to 10 mm, slices of each muscle were randomized among the eight treatment groups.

The pre-freeze-drying treatments involved spraying the meat slices with 1% by weight of ethanol containing the following (per 100g) of solution:

A–Ethanol only, no additive
B–80 mg butylated hydroxyanisole (BHA)
C–80 mg propyl gallate (PG)
D–30 mg BHA, 30 mg butylated hydroxytoluene (BHT) and 18 mg PG
E–40 mg citric acid (CA)
F–80 mg BHA + 40 mg CA
G–80 mg PG + 40 mg CA
H–30 mg BHA + 30 mg BHT + 18 mg PG + 40 mg CA

RESULTS & DISCUSSION

RESULTS were statistically analyzed by Analysis of Variance, with animals, or replicates, being considered as random selections from an infinite choice, and storage and treatments being fixed states. Percent of variance was calculated by the method of Hicks (1956).

"Tenderstretch" hanging, aging and storage temperature

Storage temperature accounts for most of the variance apart from error (42% and 47% respectively), with storage at 37°C for 90 days increasing hardness of freeze-dried beef semimembranosus by 18.2N ($P < 0.001$), with samples stored at 37 and 1°C having hardness of 103.5 and 85.3N.

"Tenderstretch" hanging gave samples with average hardness of 92.5N compared with 100.1N for meat hung conventionally. This difference was very significant ($P < 0.01$) but represents only 6.4% of the total variance.

"Tenderstretch" hanging stretches many of the muscles of the hindquarter, including M. semimembranosus and M. adductor, during the onset of rigor which increases sarcomere length in the meat, causing a decrease in shear and resulting in a more tender product (Hostetler et al., 1972). As the improvement is due to increased sarcomere length which is carried from cooked meat to freeze-dried cooked meat, no shrinkage occurring during drying, this improvement in texture is expected to be carried through to the freeze-dried product as observed.

Aging for 18 days caused an average decrease in hardness of 2.8N compared to 4 days aging, samples having average hardness of 97.7 and 94.9N for 4 and 18 days aging respectively. This is significant ($P < 0.05$) but represents only 0.7% of the total variance.

Aging results in a breakdown of the Z-line of myofibrils (Davey and Gilbert, 1969) thus decreasing the fiber strength of the meat. As with the effect of "tenderstretch" hanging, this effect is carried through to the freeze-dried product.

The interaction between aging and storage temperature was significant ($P < 0.01$, 3.4% of variance), samples stored at 37°C having hardness 21N and 14.6N above 1°C storage for 4 days and 18 days aging respectively. If cross linking of protein chains in the myofibrils is the cause of the increase in hardness, then as myofibrils are broken at the Z-line in aged meat this cross linking will not produce such a large increase in hardness as in unaged meat.

Internal temperature attained during cooking

Hardness of freeze-dried meat cooked to internal temperatures of 50, 75, 85 and 98°C and stored at 1 and 37°C for 90 days are shown in Table 1. The effects of cooking temperature were large and significant ($P < 0.001$), with increasing internal temperatures up to 75°C resulting in increased hardness, but cooking to 85°C or above resulted in decreased hardness; cooking to 98°C internal temperature gave a product with lower hardness than cooking to 50°C.

Davey and Gilbert (1974) have studied the effect of increasing temperatures on the shear values of meat and have made the following observations. Increasing temperatures between 40 and 50°C results in denaturation of myofibrillar proteins

Table 1—Texturometer hardness (Newton) of stored, freeze-dried semimembranosus cooked to various internal temperatures

Internal temp[a] (°C)	Hardness (N) after storage at[b,c] 1°C	37°C
50	91.0	93.7
75	114.1	128.2
85	108.8	116.4
98	80.8	95.4

[a] Internal temperature attained during cooking in atmospheric steam.
[b] Vacuum packed and stored for 90 days. Each figure represents the average of 16 determinations.
[c] Effects of storage temperature and cooking temperature X storage temperature interaction are not significant ($P > 0.05$).

Table 2—Effect of cooking temperature and time, and storage temperature on hardness of stored, freeze-dried beef semimembranosus

Cooking temp[a] (°C)	Hardness (N) after storage at[b] 1°C Cooking time[c] 2 hr	1°C Cooking time 4 hr	37°C Cooking time 2 hr	37°C Cooking time 4 hr
55	83.3	80.7	102.1	99.3
65	80.3	83.3	103.3	110.5
75	94.1	95.0	111.9	115.7
85	100.6	78.9	121.7	100.2

[a] Samples were packed in polyethylene bags and cooked in a water bath maintained at indicated temperature for 2 or 4 hr. Cooking temperature had a significant effect on hardness ($P < 0.05$) explaining 5.2% of total variance.
[b] Vacuum packed and stored for 90 days. Each figure represents the average of 24 determinations. Effect of storage temperature was significant ($P < 0.05$) explaining 41.8% of total variance. Interactions of cooking time and temperature with storage temperature were not significant ($P > 0.05$).
[c] There was a significant interaction of cooking time and temperature ($P < 0.05$) explaining 13.2% of total variance.

Table 3—Cooking drip loss and hardness of freeze-dried beef semimembranosus pretreated with salt solutions

Treatment[a]	Drip loss[b] (%)	Hardness (N) after storage at[c] 1°C	37°C
Control	41.7	94.2	120.0
7% NaCl + 1.2% KCl + 0.02% ascorbic acid	33.5[e]	97.4	110.0
7% NaCl + 1.2% KCl	32.5[e]	97.3	114.6
0.02% Ascorbic acid	42.0	100.1	122.3
Corning[d]	24.2[e]	97.2	105.6

[a] Solution injected to 10% of raw weight before samples cooked to 75°C internal temperature in atmospheric steam.
[b] Percentage of weight loss during cooking (average of 4 determinations).
[c] Vacuum packed and stored for 90 days. Each value represents the average of 24 determinations. Effect of treatment, storage temperature and storage temperature X treatment interaction not significant ($P > 0.05$).
[d] 14% NaCl, 6% sucrose, 0.6% $NaNO_3$ and 0.1% $NaNO_2$
[e] Values so marked are significantly different from control ($P < 0.05$, student "t").

giving rise to higher shear values. Further heating between 65 and 75°C caused shrinkage of collagen, resulting in shrinkage of meat, weight loss and increased shear. Above 75°C there was a decrease in shear as collagen was solubilized. Sarcoplasmic proteins did not appear to be related to toughness, denaturation occurring over the range of 40–80°C while increases in toughness were confined to those temperature regions mentioned.

Bouton and Harris (1972) studied the effect of cooking temperature and time on adhesion and chewiness, measures of connective tissue strength, and showed a small reduction at 60°C in 1-yr-old steers, and reductions for all age groups after 1 hr at 90°C.

The present results agree with the generally observed effects of cooking temperature on meat toughness, meat becoming drier and harder as temperature rises and finally becoming more tender as collagen is gelatinized.

Storage temperature X cooking temperature interactions resulted in large but not significant effects on hardness (Table 1).

Cooking temperature and time, and storage temperature

M. semimembranosus samples were cooked to varying internal temperatures and times inside polyethylene bags in a water bath. The time taken for the internal temperature to reach the bath temperature was around 2 hr.

Table 2 lists hardness of freeze-dried meat subjected to various cooking and storage conditions.

Storage temperature significantly affected the hardness of rehydrated meat with average hardness of 37 and 1°C storage samples being 108.1 and 86.8N. Cooking temperature also significantly affected hardness, hardness increasing with temperature. Cooking for 4 hr decreased hardness compared with 2 hr cooking by 4.4N, but this effect is not significant.

The interaction of cooking time and temperature was however significant ($P < 0.01$) with cooking for the additional 2 hr at 85°C causing a decrease in hardness of 22.4N. Heating for the additional 2 hr at 75°C or below did not result in significant reductions in hardness.

As with meat cooked to various internal temperatures, interactions involving storage temperature and cooking parameters were not significant ($P > 0.05$), indicating that while cooking temperature and time have large effects on the hardness of beef, both fresh and freeze dried, they do not affect the increase in hardness caused by high temperature storage of freeze-dried beef.

Infusion with salt solutions

Analysis of variance indicates a highly significant effect of storage temperature on meat hardness. Samples stored at 37°C for 90 days had average hardness of 114.5N, 17.3N above that for 1°C storage samples.

The solutions containing sodium and potassium chlorides decreased cooking drip loss (Table 3) as was earlier reported by Hamdy et al. (1959). Hamdy et al. (1959) also noted increased taste panel scores for tenderness and juiciness in modified meat cooked before freeze drying. Although significant only at the 10% level because of interactions between replicates and treatments, treatment effects were large. Treatment with sodium and potassium chlorides or corning solution reduced the extent of hardening of stored, freeze-dried meat in comparison to untreated controls.

Treatment X storage temperature interactions were not significant because of higher order interactions with replicates, but the large effects of salts, particularly corning solution, in preventing deterioration of texture during high temperature storage are noteworthy. For example corned samples stored at 37°C had hardness 8.4N greater than 1°C storage samples, but untreated control samples increased in hardness by 25.8N. Because of these and similar observations it was thought that

further work on the components of corning mixture would be worthwhile.

Corning components

The effects of the various components were studied following injection of the muscle with 10% by weight of aqueous solutions followed by cooking and freeze drying. Sodium chloride (140 g/liter), concentrations similar to those used in the previous experiment, had large and varying effects on cooking drip loss and hardness of stored, freeze-dried beef (Table 4), with treatment and storage temperature X treatment interaction accounting for 19 and 7% of total variance respectively.

Injection of an aqueous solution of sucrose (60 g/liter) had no effect on hardness of stored, freeze-dried beef semimembranosus but samples so treated did not show an increase in hardness on storage for 90 days at 37°C over samples stored at 1°C. Untreated control samples when stored at 37°C increased in hardness by 15.3N in comparison to 1°C storage. Sugars have been used to preserve the viability of bacterial suspensions during freeze drying and subsequent storage but little is known of the mechanism of this protective effect (Fry, 1966).

Treatment with a solution of sodium chloride (140 g/liter) gave a product with lower hardness (92.1N) than untreated controls stored similarly (112.8N). This may be related to the significantly lower drip loss of meat treated with sodium chloride (Table 4) also previously noted by Shults et al. (1972). Increased water-holding capacity (WHC) of meat associated with increased pH is known to have a favorable effect on tenderness (Hamm, 1960; Bouton et al., 1973), and possibly increased WHC caused by addition of sodium chloride will also increase tenderness.

Sodium chloride treated meat also had a small increase in hardness on high temperature storage compared to control samples. This may also be related to increased WHC, as Penny et al. (1963) observed that raw beef of high ultimate pH, obtained by pre-slaughter injection with adrenaline, gave freeze-dried beef which deteriorated more slowly on storage than control. There may be some other mechanism also responsible as sucrose treatment did not affect drip loss of beef semimembranosus, but had an even larger effect in preventing hardening caused by high storage temperature (Table 4).

Addition of potassium nitrate (6 g/liter) caused a decrease in average hardness of 10.6N compared to control but there was no significant difference in the high temperature induced hardening of control and treated samples.

Sodium nitrite at the concentration of 1 g/liter in the added solution had no effect on storage induced hardness or average hardness of freeze-dried beef compared to untreated control.

Evaporative vs pre-freezing and rehydration with papain, pyrophosphate and citrate

Analysis of variance data and percent of variance for the effects of storage temperature, freezing method and rehydration solution are shown in Table 5.

Storage temperature had a significant effect on hardness of freeze-dried beef, with samples stored at 37° and 1°C having average hardness of 84.7N and 71.3N respectively.

Evaporative freezing and pre-freezing in still air at −18°C gave products of similar hardness, 79.4 and 79.9N respectively, but there was a very significant replicate X freezing method interaction. This is probably due to randomizing the sides among freezing methods. De Felice et al. (1965) found very large differences in hardness between the sides of a steer. As the difference between freezing methods are small and less than the variation within an animal, it appears that any effect of evaporative freezing will be unimportant.

Tuomy et al. (1962) reported an adverse effect of evaporative freezing on texture of beef: pre-frozen meat being superior in juiciness, tenderness and cutability. De Felice et al. (1965) found that evaporative freezing considerably increased hardness of freeze-dried beef, but in conjunction with overdrying gave a product of similar hardness to pre-frozen samples. However, evaporative freezing followed by either normal drying or overdrying yielded products with increased juiciness.

Table 4—Effect of treatment with corning salts on cooking drip loss, hardness and increase on hardness in storage of freeze-dried beef semimembranosus

Treatment[a]	Drip loss[b] (%)	Hardness[c] (N)	$H_{3_7} - H_1$[d] (N)
Control	30.4	112.8	15.3
Sucrose—6%	33.9[e]	113.3	0.1[e]
NaCl—14%	22.5[e]	92.1[e]	4.7[e]
KNO_3—0.6%	33.9[e]	102.2	17.1
$NaNO_2$—0.1%	32.6	120.8	21.2

[a] Injected to 10% of raw weight. All samples cooked by heating in a water bath at 75°C for 2 hr.
[b] Percentage of weight lost during cooking. Each figure is average of 4 determinations.
[c] Each value represents the average of 56 determinations derived from 4 replicates stored at both 1 and 37°C.
[d] $H_{3_7} - H_1$ is difference in average hardness of samples stored for 90 days at 37 and 1°C.
[e] Figures so marked are significantly different from control ($P < 0.05$, Student "t").

Table 5—Analysis of variance results and percent of total variance for effects of storage temperature, freezing method and rehydration in papain, pyrophosphate or citrate solution on hardness

Source of variation	df	Significance	% Variance
Storage temp[a]	1	<0.01	12.2
Papain conc (P)[b]	3	<0.001	31.9
Citrate conc (C)[c]	3	NS[e]	–
Freezing method (F)[d]	1	NS[e]	–
C X P	9	<0.01	0.7
P X F	3	<0.001	4.4
Remainder	384	–	50.7

[a] 37 or 1°C for 90 days
[b] 0, 5, 20 or 100 ppm in rehydrating solution
[c] 0, 0.5 or 1% sodium citrate or 0.5% tetrasodium pyrophosphate in rehydrating solution
[d] Pre-freezing at −18°C in still air or evaporative freezing inside freeze-dryer chamber
[e] NS—not significant $P > 0.05$

Table 6—Texturometer hardness and taste panel texture scores for freeze-dried, beef top round rehydrated in papain solution

Papain conc (ppm)	Hardness[a] (N)	Taste panel score[b] Juiciness	Tenderness	Overall texture
0	90.3[c]	4.5[c]	4.5[c]	4.5[e]
5	86.5[c]	4.4[c]	4.7[c,d]	4.6[c]
20	79.1[d]	5.1[d]	5.3[d]	5.2[d]
100	56.1[e]	5.6[e]	6.1[e]	5.6[d]

[a] Each value represents the average of 16 determinations, from each of 4 replicates
[b] 9-point hedonic ratings (9 = excellent, 1 = very poor), N = 5.
[c,d,e] Figures in any column with different superscripts are significantly different by Duncan Multiple Range Test ($\alpha < 0.05$)

Increasing concentrations of papain in the rehydrating solution significantly decreased hardness as shown in Table 6.

Taste panel results are also summarized in Table 6 and indicate increasing scores for juiciness, hardness and overall texture with increasing papain concentration.

Pyrophosphate on addition to fresh meat caused an increase in pH, resulting in increased WHC (Shults et al., 1972). Bouton et al. (1973) have shown that increased pH or WHC increased both objective and subjective tenderness. The present experiment showed no improvement in taste panel or texturometer texture on rehydration, either in 0.5% pyrophosphate or 0.5 and 1% sodium citrate solution. These conditions may be similar to those of Hamm and Deatherage (1960) who found that increase in pH after cooking caused a much smaller increase in WHC than when pH was adjusted prior to heating of the muscle.

The significant interaction between papain concentration and freezing method is unexplained.

Antioxidants

Storage temperature accounted for most of the variance not due to error or replicate animals with storage at 37°C giving samples of average hardness 129.8N, compared with 1°C storage of 11.6.6N.

Antioxidants had no significant effect on hardness of stored freeze-dried beef. This may be due to poor penetration of antioxidants into the meat slices or the ineffectiveness of antioxidants in preventing autoxidation of bound lipids (Chipault and Hawkins, 1971) a reaction known to lead to crosslinking of proteins (Andrews et al., 1965).

The citric acid X storage temperature interaction was significant ($P < 0.05$), with meat samples sprayed to 1% of cooked weight with a solution of 40 mg/100 ml citric acid in ethanol having hardness 10.5N higher for 37°C storage than at 1°C, while the difference between the two storage temperatures was 16N for untreated controls. This appears not to be due to synergism with the antioxidant, as the storage temperature X antioxidant X citric acid and antioxidant X citric acid interactions were not significant. It may instead be due to buffering action of citric acid, lowering the pH of meat. The rate of nonenzymic browning in meat extracts was slowed as pH was lowered (Sharp and Rolfe, 1958). Browning-type reactions, among other factors, could cause toughening in freeze-dried meats. However, a more likely explanation is that citric acid chelates metal ions present in meat, preventing them from acting as catalysts in the oxidation of lipids. This catalytic action of metal ions is important in freeze-dried meats due to the low relative humidity conditions causing the ions to exist in unhydrated, catalytic forms (Labuza et al., 1966).

Matyniak et al. (1971) reported that ethylenediaminetetracetic acid (EDTA), a better chelator than citric acid, improved the stability of freeze-dried chicken and Sweet (1973) reported that EDTA, when used alone, increased the induction period for onset of rancidity in fresh fish more than any of the common antioxidants used alone.

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Ms received 7/3/75; revised 12/5/75; accepted 12/8/75.

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FATNESS, RATE OF CHILLING AND TENDERNESS OF LAMB

ABSTRACT

40 lambs were selected to vary in amount of finish for use in determining the mechanism by which fatness affects tenderness. Lambs with thick (n = 10), intermediate (n = 20) or thin (n = 10) finish were slaughtered, chilled at 1 ± 1°C and samples obtained from three muscles for chemical, histological, physical and organoleptic analyses. Subcutaneous fat was removed from one side of each carcass in the intermediate finish group to facilitate comparisons on a within-carcass basis. The data suggest that lamb carcasses which have increased quantities of fat (subjectively determined via live evaluations of finish or objectively determined via subcutaneous fat thickness measurements and marbling scores): (a) chill more slowly; (b) maintain muscle temperatures conducive to autolytic enzyme degradation for greater periods of time postmortem; (c) sustain less shortening of sarcomeres; (d) have muscles with lower ultimate pH values; (e) have less perceptible or softer connective tissue; and (f) are more tender than lamb carcasses which have limited quantities of fat. The data support the hypothesis that deposition of increased quantities of subcutaneous or intramuscular fat (particularly in carcasses with limited quantities of subcutaneous finish) increases tenderness via changes in postmortem chilling rate. An increased quantity of fat decreases the rate of temperature decline (either by insulation or via an increase in carcass mass), enhances the activity (or increases the duration of active proteolysis) of autolytic enzymes in muscle, lessens the extent of myofibrillar shortening and thereby increases the ultimate tenderness of cooked meat from a fatter carcass.

INTRODUCTION

THERE IS NOW ample evidence that changes in the myofibrillar component of muscle which occur pre-rigor (i.e., during the period between slaughter and the full development of rigor mortis) can markedly influence the tenderness of the resulting meat (Newbold and Harris, 1972). Locker (1960) described the relationship between tenderness and extent of postmortem muscle shortening. If the muscles of the carcass are provoked to shorten, as by early rapid chilling, they will shorten to the fullest extent allowed by skeletal restraints, lose their extensibility and ultimately become fixed in that state of contraction concomitant with development of rigor mortis. Postmortem contraction of myofibrils can be controlled by allowing rigor mortis to develop at temperatures which minimize shortening or by physically restraining the muscle from shortening (Newbold and Harris, 1972).

The amount of muscle shortening decreases as the period between slaughter and exposure to cold is extended (Marsh and Leet, 1966). Chilling at 0–1°C produces cold shortening (Locker and Hagyard, 1963) which can reduce the tenderness of certain muscles in the intact carcass (Bouton et al., 1973b). Conditioning of carcasses by pre-rigor chilling at 15–20°C lessens the myofibrillar toughness of beef (Smith et al., 1971; Parrish et al., 1973; Bouton et al., 1973a) and of lamb (Cook and Langsworth, 1966; Marsh et al., 1968; McCrae et al., 1971; Bouton et al., 1973b; Smith et al., 1976). Marsh et al. (1968) recognized that cooling rate is determined not only by ambient temperature, humidity and air velocity, but also by the size of the cooling body and the depth below overlying tissue of the particular muscle being considered. The results of Wenham et al. (1973) suggest that larger carcasses (mature rams) chill more slowly than smaller carcasses and thus are more tender. Wenham et al. (1973) further observed that mature ewe carcasses sustained greater toughening from early freezing than did lamb carcasses. The latter result is presumed to occur because the blocky, thick and fat lamb carcasses cool more slowly than do the angular, thin and lean mature ewe carcasses. Marsh and Leet (1966) concluded that it would require only a slightly greater thickness of fat, the proximity of bone, or a protective shielding (by overlying tissue) of a part of a muscle from cold, moving air to allow muscles which are fixed absolutely in length by attachments to the skeletal framework to sustain cold shortening in part of their length.

Early animal production studies (recently reviewed by Smith and Carpenter, 1975) indicated that fatter animals usually produced meat that was more tender than that from leaner animals. Because fatter animals tend to deposit greater quantities of marbling, these early studies were interpreted to mean that increased deposition of intramuscular fat was associated with increased palatability of cooked meat. Research during the last 50 yr has failed to clearly elucidate the mechanism by which marbling affects tenderness; and, in fact, has not clearly shown that increased fatness is necessary to achieve acceptable or exceptional eating satisfaction (Smith and Carpenter, 1975). In light of recent findings regarding the importance of the time-temperature history of the carcass during the pre-rigor period and its marked effect on muscle tenderness (Newbold and Harris, 1972), the role of fatness in altering the rate of carcass chilling needs to be thoroughly investigated.

In studies of the palatability of goat meat, Pike (1974) observed certain thinly-finished and light-weight carcasses which produced very tough cooked meat and muscles with very short sarcomeres. The latter results suggest that carcass weight and/or fatness can affect tenderness via the cold shortening phenomenon. Cross et al. (1972) and Reagan (1974) reported significant ($P < 0.01$) correlations between intramuscular fat content and sarcomere length in lamb and beef longissimus muscles, respectively, suggesting that marbling might also be related to tenderness via its insulatory effect in reducing the severity of cold shortening induced by low temperature chilling. These reports led to the hypothesis that subcutaneous and/or intramuscular fat could affect the tenderness of muscles by insulating the muscle fibers during postmortem chilling, changing the rate of temperature decline and thereby decreasing the extent of cold shortening. The present study was designed to identify and elucidate such mechanisms because, if fatness increases tenderness via its effect in lessening the rate of postmortem chilling, then there are other, more practical and less costly, procedures for enhancing meat tenderness than by increasing the amount of subcutaneous fat cover or the extent of intramuscular fat deposition.

EXPERIMENTAL

40 CROSSBRED (fine wool × medium wool) lambs were selected from a commercial feedlot on the basis of maturity and fatness. Two trained evaluators physically handled each lamb in the manner described by the

Table 1—Carcass characteristics for lambs in each finish group

Trait	Finish group		
	Thick	Intermediate	Thin
Carcass weight, kg	28.6[a]	21.5[b]	16.8[c]
USDA quality grade[d]	13.5[a]	11.4[b]	8.6[c]
USDA yield grade	4.1[a]	3.0[b]	2.1[c]
Fat thickness-12th rib, mm	7.1[a]	3.3[b]	1.1[c]
Longissimus muscle area, cm^2	14.9[a]	13.1[ab]	10.6[b]
USDA marbling score[e]	19.6[a]	15.1[b]	9.5[c]

[a,b,c] Means in the same horizontal row bearing different superscripts are different ($P < 0.05$).

[d] Coded as follows: Avg Prime = 14, Avg Choice = 11, Avg Good = 8, etc.

[e] Coded as follows: Abundant = 29, Mod. abundant = 26, Sl. abundant = 23, etc.

USDA (1960) to determine degree of finish, to estimate the thickness of subcutaneous fat over the longissimus muscle in the 12–13th rib region and to determine physiological maturity. Live lambs were selected to exhibit thick, intermediate or thin finish, to have >7.5 mm, 2.5–7.5 mm or <2.5 mm of estimated fat thickness and to be approximately 5–9 months of age at the time of slaughter. There were 10 lambs with thick finish, 20 lambs with intermediate finish and 10 lambs with thin finish.

The live lambs were transported to the Texas A&M University meat laboratory and slaughtered in groups of four lambs each. One lamb with thick finish, two lambs with intermediate finish and one lamb with thin finish constituted a slaughter group. Each group of four lambs was slaughtered with sufficient dispatch to assure that the entire group entered the cooler (1 ± 1°C) within 40 min following death. The right side of each lamb carcass in the intermediate finish group (n = 20) was carefully trimmed to remove the subcutaneous fat from the rack, loin, sirloin and leg. Fat was removed from the dorsal midline to a point approximately 10 cm lateral to the midline over the rack, loin and sirloin. All of the subcutaneous fat was removed from the inside and outside cushion portions of the leg. The trimming operation was performed immediately prior to chilling and the carcasses were not split. All subcutaneous fat and fell membrane remained intact on lambs with thick finish, lambs with thin finish and on the left side of each lamb with intermediate finish. Recorders continuously monitored temperature decline during postmortem chilling via thermocouples placed in the center of the longissimus muscle opposite the 13th rib. One thermocouple per carcass was used for lambs with thick or thin finish; two thermocouples per carcass were used for lambs with intermediate finish.

Following 72 hr in the cooler, each carcass was evaluated for USDA grade factors, longissimus muscle area and marbling score. The carcasses were then separated into wholesale cuts and rib chops, loin chops and leg steaks were obtained for subsequent chemical, histological and palatability analyses. Measurements of pH were obtained by use of a Corning Model 12 pH meter on 5-g samples of longissimus or biceps femoris muscle which had been blended with 25 ml of distilled-deionized water. Sarcomere length was determined by measuring the length of 10 sarcomeres from each of 25 myofibrils from each muscle at a magnification of 1000× by use of a filar micrometer. Samples were prepared for sarcomere measurement by homogenizing 3g of longissimus or biceps femoris muscle with 35 ml of 0.25M sucrose solution for 40 sec.

Two complete sets of palatability samples (loin chops and leg steaks) were prepared, wrapped in polyethylene-coated paper and frozen for subsequent cooking. Chops and steaks from each carcass (from each side of lambs with intermediate finish) were individually thawed at 3°C for 12 hr, oven-broiled (Cover et al., 1962) in preheated 177°C gas ovens (one sample per oven) to an internal temperature of 75°C and divided into individual portions. Loin chops were weighed before and after cooking and the time (in minutes) required to reach the desired degree of doneness was recorded. Cooked samples of three muscles (longissimus, biceps femoris and semimembranosus) were scored by a six-member trained sensory panel by use of the tenderness profile system described by Cover and Hostetler (1960). Scores were assigned for juiciness, tongue and cheek pressure, tooth pressure, mealiness, adhesion, fragmentation, connective tissue amount and connective tissue softness. Shear force values were obtained for longissimus muscles in duplicate on 1.3 cm cores which were removed after the cooked samples had cooled to 25°C.

Additional chops and steaks from each carcass (from each side of lambs with intermediate finish) were individually thawed at 3°C for 12 hr, oven-broiled (Cover et al., 1962) in preheated 177°C gas ovens (one sample per oven) to an internal temperature of 80°C and divided into individual portions. Cooked samples of all three muscles were scored by an eight-member trained sensory panel for three tenderness traits (muscle fiber tenderness, connective tissue tenderness and overall tenderness) in the manner described by Cross et al. (1973). Shear force values were obtained in duplicate from 1.3 cm cores (removed after the cooked samples had cooled to 25°C) by use of the Warner-Bratzler shear machine.

The data were analyzed by the use of statistical procedures described by Snedecor and Cochran (1967), Duncan (1955) and Kramer (1956). Main effects for analyses of variance included finish groups, subcutaneous fat thickness groups and marbling score groups. Mean separation analyses were performed if a main effect was significant at the 0.05 probability level.

RESULTS & DISCUSSION

CARCASS CHARACTERISTICS for lambs in each finish group are presented in Table 1. Carcasses from lambs in the thick finish group were ($P < 0.05$) heavier, more muscular, fatter and higher grading than were lambs in the thin finish group. It should be noted that carcass weight and fatness are confounded in these data. Since fat carcasses with exactly the same volume of bone and muscle as lean carcasses would, of necessity, be heavier, the weight differential observed here was not considered to be of major consequence. Increased fatness may decrease chilling rate by its insulatory capacity or by its effect in increasing mass; in either case, fatter lambs would chill more slowly.

Since the original hypothesis was predicated upon differential rates of postmortem chilling among carcasses differing in fatness or finish, mean internal temperatures were collected for longissimus muscle from carcasses in each finish group (Table 2). Carcasses in the thick finish group had higher mean internal temperatures in the longissimus muscle than did carcasses in the intermediate finish group after 0, 1, 2, 3 and 4 hr of chilling. Lambs with intermediate finish chilled more slowly than did carcasses with thin finish, as evidenced by significant differences in longissimus muscle temperature at 1, 2 and 3 hr postmortem. These data support the contention that fatter (or heavier) lambs chill more slowly than leaner (or lighter) lambs ($P < 0.05$) during the first 4 hr immediately postmortem. Field et al. (1970) reported that higher longissimus muscle temperatures at 1 hr postmortem were associated with decreased shear force requirements ($r = -0.61$) and with increased tenderness ($r = 0.81$) in yearling bull carcasses. In the present study, the correlation between temperature at the center of the longissimus muscle at 1 hr postmortem and shear force requirement for the cooked longissimus muscle was -0.67 ($P < 0.05$).

Mean internal temperature of the longissimus muscle (Table 2) attained 1°C after 8.1, 5.9 and 5.6 hr of chilling in thick, intermediate and thin finish groups, respectively. In lamb carcasses chilled at 0–1°C, Bouton et al. (1973b) found that the temperature in the center of the longissimus reached 1°C in 6.4 hr and concluded that chilling at 0–1°C produced cold shortening which reduced the tenderness of the larger muscles in the sheep carcass. Whether the 2.5 hr (8.1 minus 5.6 hr) chilling time differential between lamb carcasses with thick vs thin finish is sufficient to effect measurable changes in tenderness is not presently known. Marsh et al. (1968) reported that very significant toughness develops in the longissimus muscles of lamb carcasses exposed to low temperatures within about 16 hr of slaughter. The period of 16 hr of high temperature for chilling-conditioning is generally supported by McCrae et al.

(1971) for lamb and by Smith et al. (1971) for beef. Dutson et al. (1975) reported that carcass temperature in the first 12 hr postmortem is important in determining beef muscle tenderness.

Sarcomere lengths and pH values for two muscles from each carcass in the three finish groups are presented in Table 3. Carcasses in the thick and intermediate finish groups, had the longest sarcomeres, while carcasses in the thin finish group had the shortest ($P < 0.05$) sarcomeres. Cook and Langsworth (1966) found that minimum shortening of ovine muscle occurred at chilling temperatures between 5 and 20°C while maximum shortening was noted at chilling temperatures of 0 or 40°C. In the present study, lambs with thick, intermediate and thin finish had internal longissimus muscle temperatures between 5 and 20°C for 4.5, 3.1 and 2.1 hr, respectively. Bouton et al. (1973b) concluded that myofibrillar toughness of cooked meat is virtually independent of sarcomere length for muscles with sarcomeres which are longer than about 1.8 μm and that lamb carcasses chilled at 15–16°C had a few muscles with sarcomeres much shorter than about 1.8 μm. The latter researchers reported mean sarcomere lengths for longissimus, biceps femoris and semimembranosus muscles of 1.65, 1.62 and 1.71 μm, respectively, for carcasses chilled at 0–1°C and 1.77, 1.73 and 1.78 μm, respectively, for carcasses chilled at 15–16°C (Bouton et al., 1973b). Sarcomere lengths in the present study (Table 3) were of comparable magnitude to those from lambs conditioned at high temperature in the Bouton et al. (1973b) study.

Carcasses in the thin finish group had significantly ($P < 0.05$) higher pH values than did lambs in the thick finish group (Table 3). The rate of fall of pH depends upon muscle temperature (Newbold and Harris, 1972) and pH falls more rapidly at 1°C than at 5°C during the first few hours postmortem (Cassens and Newbold, 1967). Rate of pH decline was not determined in the present study, but ultimate pH of both muscles was lowest ($P < 0.05$) for lambs in the thick finish group. Field et al. (1970) found that pH value at 1 hr postmortem was closely associated with beef tenderness; the higher the pH, the higher the shear force value ($r = 0.73$) and the lower the tenderness rating ($r = -0.68$). However, Bouton et al. (1971) has shown that the tenderness of sheep muscle is greater with higher ultimate pH. Results of the present study agree with those of Field et al. (1970) with regard to less tender muscles having a higher pH value.

Ratings for juiciness and tenderness of three muscles from each lamb carcass in the three finish groups are presented in Table 4. Juiciness ratings for longissimus and biceps femoris muscles did not differ among samples from lambs in the three finish groups, despite rather large differences in amount of intramuscular fat (Table 1). Samples from lambs with thick finish evoked less pressure to tongue, cheek and tooth, were more mealy, had less intrasample adhesion, fragmented more readily, had lesser quantities of connective tissue and characteristically softer connective tissue than did samples from carcasses with thin finish ($P < 0.05$ for 19 of 21 comparisons). Sensory panel ratings which are indicative of myofibrillar tenderness-toughness (tongue-cheek pressure, tooth pressure, mealiness and fragmentation) closely parallel results of sarcomere length determinations (Table 3). For example, longissimus muscles from lambs with thick vs intermediate finish did not differ in sarcomere length and did not differ significantly in tongue-cheek pressure, mealiness or fragmentation. Conversely, longissimus muscles from carcasses with thick vs thin finish differed ($P < 0.05$) in sarcomere length and differed ($P < 0.05$) in tongue-cheek pressure, tooth pressure, mealiness and fragmentation. Relationships between sarcomere length and tenderness of similar magnitude have been reported by Cook and Langsworth (1966).

Sensory panel ratings which are indicative of background effects on tenderness (adhesion, connective tissue amount and connective tissue softness) revealed significant ($P < 0.05$) differences between lambs with thick vs intermediate finish in only 2 of 9 comparisons. Similar comparisons between lambs with intermediate vs thin finish and carcasses with thick vs thin finish indicate significant ($P < 0.05$) differences in 8 of 9 and 8 of 9 comparisons, respectively. These results closely parallel results for internal muscle temperatures (Table 2), sarcomere length (Table 3) and pH (Table 3). Sarcomere length should not be related to measures of connective tissue, and the fact that such a corollary was found may merely reflect the inability of sensory panel members to isolate and quantify this part of the tenderness profile analysis. Conversely, the relationships between pH and postmortem muscle temperature with measures of connective tissue may indicate a mechanism related to autolytic enzyme activity.

Lysosomes are small cytoplasmic organelles, which contain many acid hydrolases within the lysosomal membrane (Hirsch and Cohn, 1964). Activity of lysosomal acid hydrolases is increased by many treatments including low pH and high temperatures (Weisman, 1964; Stagni and De Bernard, 1968) which labilize the lysosomal membrane. Both of these conditions are fulfilled in the present study (low pH for the more tender muscles, Tables 3 and 4; high temperature for the more tender muscles, Tables 2 and 4). Moeller et al. (1976) reported that holding of beef carcasses at elevated temperature for 12 hr postmortem enhanced the disruption of the lysosomal

Table 2—Internal temperatures for longissimus muscles from carcasses in each finish group

Time postmortem (hr)	Temperature (°C)		
	Finish group		
	Thick	Intermediate	Thin
0.0	30.7[a]	26.3[b]	26.0[b]
0.5	22.6[a]	19.8[ab]	16.7[b]
1.0	19.0[a]	15.6[b]	11.5[c]
2.0	13.8[a]	10.8[b]	6.5[c]
3.0	10.7[a]	7.3[b]	3.8[c]
4.0	7.9[a]	3.8[b]	2.4[b]
5.0	5.4[a]	2.5[a]	1.7[a]
6.0	3.6[a]	0.9[a]	0.7[a]

[a,b,c] Means in the same horizontal row bearing different superscripts are different ($P < 0.05$).

Table 3—Sarcomere lengths and pH values for two muscles from carcasses in each finish group

Trait	Finish group		
	Thick	Intermediate	Thin
Sarcomere length, longissimus (μm)[c]	1.78[a]	1.78[a]	1.70[b]
Sarcomere length, biceps femoris (μm)[c]	1.86[a]	1.83[ab]	1.78[b]
pH value, longissimus[c]	5.60[a]	5.70[b]	5.75[b]
pH value, biceps femoris[c]	5.69[a]	5.71[a]	5.80[b]

[a,b] Means in the same horizontal row bearing different superscripts are different ($P < 0.05$).

[c] Sarcomere length and pH determinations were obtained from fresh muscle samples taken from the carcasses 72 hr postmortem.

Table 4—Ratings for juiciness and tenderness for three muscles from lamb carcasses in each finish group

Trait	Longissimus (L) Finish group			Biceps femoris (BF) Finish group			Semimembranosus (SM) Finish group		
	Thick	Int.	Thin	Thick	Int.	Thin	Thick	Int.	Thin
Juiciness	6.1[a]	6.1[a]	6.0[a]	5.4[a]	5.1[a]	5.0[a]	5.1[ab]	5.3[a]	4.8[b]
Tongue-cheek pressure	7.4[a]	7.1[ab]	6.8[b]	6.9[a]	6.3[b]	6.2[b]	6.4[a]	6.1[ab]	5.9[b]
Tooth pressure	7.0[a]	6.5[b]	5.2[c]	6.9[a]	5.7[b]	5.0[c]	5.6[a]	5.2[ab]	4.5[b]
Mealiness	3.5[a]	3.4[a]	2.9[b]	3.6[a]	3.2[b]	2.6[c]	2.9[a]	2.8[a]	2.6[a]
Adhesion	7.3[a]	6.8[b]	6.0[c]	7.2[a]	5.9[b]	5.3[c]	5.8[a]	5.5[a]	4.8[b]
Fragmentation	7.2[a]	6.8[a]	5.7[b]	7.2[a]	5.8[b]	5.2[c]	5.8[a]	5.5[a]	4.7[b]
Connective tissue amount	8.5[a]	8.3[a]	7.6[b]	8.3[a]	8.0[a]	7.4[b]	7.7[a]	7.7[a]	7.3[b]
Connective tissue softness	8.5[a]	8.4[a]	7.7[b]	8.3[a]	8.1[a]	7.5[b]	7.7[a]	7.6[a]	7.2[a]

[a,b,c] Means on the same horizontal row and for the same muscle bearing different superscripts are different ($P < 0.05$).

membrane and resulted in a significant increase in the free activity of β-glucuronidase and cathepsin C (both of which are lysosomal enzymes). Furthermore, Dutson et al. (1971) reported that esterase activity in pork muscle decreased markedly between 45 min and 3 hr postmortem, possibly as a result of either the rapid drop in pH or the cooling of the carcass. Data of the present study (Table 2) reveal that autolytic enzyme activity could have continued for a considerably longer period in lambs with thick finish because postmortem temperature decline was less rapid than that evidenced for carcasses with intermediate or thin finish.

Tenderness evaluations and cooking data for three muscles from each lamb carcass in the three finish groups are presented in Table 5. Lambs with thick or intermediate finish had longissimus, biceps femoris and semimembranosus muscles which were more tender than corresponding muscles from lambs with thin finish ($P < 0.05$ in 23 of 26 comparisons). Differences in tenderness between muscles from lambs with thick vs intermediate finish were much less definitive (differences were significant in only 5 of 13 comparisons). Bouton et al. (1973b) found that shear force values decreased exponentially as sarcomere length increased. In the latter study, decreases in sarcomere length of 0.23, 0.13 and 0.16 μm for longissimus, biceps femoris and semimembranosus muscles, respectively, were associated with increases of 104, 93 and 135% in shear force requirements (Bouton et al., 1973b). The data of the present study reveal that decreases of 0 and 0.08 μm (longissimus) or 0.03 and 0.08 μm (biceps femoris) were associated with increases of 33 to 63% (longissimus) or 2 to 22% (biceps femoris) in shear force requirements (Table 5).

Whereas Bouton et al. (1973b) attributed increases (93–135%) in tenderness of lamb from carcasses chilled at high (15–16°C) temperatures to large (0.13–0.23 μm) increases in sarcomere length, the data of the present study indicate that increased tenderness (2–63%) resulted from factors other than (perhaps, in addition to) changes in sarcomere length (0–0.08 μm). Dutson et al. (1975) reported that delayed chilling (for 12 hr) of beef carcasses resulted in significant increases in tenderness and sarcomere length. However, Dutson et al. (1975), Hostetler et al. (1975) and Smith et al. (1971) have reported significant increases in beef tenderness for carcasses chilled at elevated temperatures which were not associated with significant changes in sarcomere length. Both Smith et al. (1971) and Dutson et al. (1975) attributed a part of the increase in tenderness associated with the use of elevated chilling temperatures to increases in autolytic enzyme activity as well as to reductions in cold shortening. Further evidence relative to increased autolytic enzyme activity being a factor in increased tenderness of high-temperature conditioned beef carcasses has been reported by Moeller et al. (1976).

Cooking data in Table 5 reveal that longissimus muscles from lambs with thick finish sustained greater cooking losses

Table 5—Tenderness ratings, shear force values and cooking data for three muscles from lamb carcasses in each finish group

Trait	Longissimus (L) Finish group			Biceps femoris (BF) Finish group			Semimembranosus (SM) Finish group		
	Thick	Int.	Thin	Thick	Int.	Thin	Thick	Int.	Thin
Tenderness rating									
Muscle fiber	6.6[a]	6.6[a]	5.4[b]	7.3[a]	6.2[b]	5.4[c]	5.9[a]	5.8[a]	4.8[b]
Connective tissue	6.7[a]	6.6[a]	5.7[b]	6.6[a]	5.6[b]	5.2[b]	6.0[a]	5.6[a]	4.5[b]
Overall	6.2[a]	6.0[a]	5.0[b]	6.7[a]	5.6[b]	4.7[c]	5.5[a]	5.2[a]	4.0[b]
Shear force value									
Leg (kg)	–	–	–	4.9[a]	5.0[a]	6.0[b]	5.8[a]	7.2[b]	7.9[b]
Loin (kg)	4.6[a]	6.1[b]	7.5[c]	–	–	–	–	–	–
Rib (kg)	7.8[a]	8.6[ab]	10.8[b]	–	–	–	–	–	–
Cooking data									
Weight loss (%)	20.5[a]	19.6[ab]	17.9[b]	–	–	–	–	–	–
Cooking time (min)	34.1[a]	29.1[b]	28.7[b]	–	–	–	–	–	–

[a,b,c] Means on the same horizontal row and for the same muscle bearing different superscripts are different ($P < 0.05$).

than did muscles from lambs with thin finish. Increased cooking losses for muscles from fatter lambs may reflect the lower pH (Table 3) and thus the lower water holding capacity of such muscles or may have resulted from greater rendering of fat during cookery.

Juiciness ratings, tenderness scores, shear force values and sarcomere lengths for three muscles from each lamb carcass in four subcutaneous fat thickness groups are presented in Table 6. Finish groups compared in Tables 2, 3, 4 and 5 were identified prior to slaughter and were not based on exact measures of fatness. To more definitively describe effects of measured fat thickness differences on tenderness, the complete population (n = 40) of carcasses was divided into quartiles (n = 10) according to thickness of subcutaneous fat opposite the longissimus muscle at the 12th rib. Lambs in quartile I (5.2–10.2 mm of fat) were more tender ($P < 0.05$) than lambs in quartile II, III and IV in 8 of 15, 11 of 15 and 15 of 15 comparisons, respectively. Lambs in quartile II (3.0–5.1 mm of fat) were more tender ($P < 0.05$) than lambs in quartiles III and IV in 5 of 15 and 12 of 15 comparisons, respectively. Lambs in quartile III (1.8–3.0 mm of fat) were more tender ($P < 0.05$) than lambs in quartile IV (0.7–1.5 mm of fat) in 9 of 15 comparisons. These data suggest that increased deposition of fat opposite the longissimus muscle at the 12th rib is associated with increased tenderness of the longissimus, biceps femoris and semimembranosus muscles, but that the relationship is not linear. Bell (1939) reported that leg roasts from fatter lambs were more tender than those from lean lambs, but that the degree of difference was not a result of degrees of difference in separable fat content.

As was previously stated, a need exists to elucidate the mechanism by which fatness affects tenderness and to determine why fatness generally, but not perfectly, relates to tenderness of cooked muscle. The data of this study (Table 6) reveal significant decreases in tenderness between adjacent fat thickness quartiles in 8, 5 and 9 of 15 comparisons between quartiles I and II, II and III, and III and IV, respectively. Sarcomere length did not differ between quartiles I and II or II and III for either muscle (Table 5) but differed ($P < 0.05$) between quartiles III and IV for both muscles. Perhaps the reason that sarcomere length and tenderness are not perfectly related is that differential rates of enzymatic proteolysis occur among carcasses causing changes in tenderness that are not reflected in histological measurements. Smith et al. (1971) sug-

Table 6—Juiciness ratings, tenderness scores, shear force values and sarcomere lengths for three muscles from lamb carcasses in each subcutaneous fat thickness or marbling score group

	Subcutaneous fat thickness Quartile[d]				Marbling score Quartile[e]			
Trait	I	II	III	IV	I	II	III	IV
Juiciness								
Longissimus	6.1[a]	6.2[a]	6.1[a]	5.9[a]	6.1[a]	6.2[a]	6.0[a]	6.0[a]
Biceps femoris	5.5[a]	5.4[ab]	5.1[ab]	4.8[b]	5.5[a]	5.2[a]	4.5[b]	5.1[ab]
Semimembranosus	5.5[a]	5.1[ab]	5.2[ab]	4.7[b]	5.1[a]	5.4[a]	5.1[a]	5.0[a]
Tooth pressure								
Longissimus	7.1[a]	6.5[ab]	6.4[b]	5.2[c]	6.9[a]	6.9[a]	6.3[a]	5.1[b]
Biceps femoris	6.9[a]	6.4[a]	5.4[b]	4.8[b]	6.8[a]	6.3[a]	5.4[b]	5.0[b]
Semimembranosus	6.0[a]	4.9[bc]	5.2[b]	4.4[c]	5.3[a]	5.5[a]	5.2[ab]	4.5[b]
Fragmentation								
Longissimus	7.3[a]	6.3[b]	6.8[ab]	5.5[c]	7.1[a]	7.1[a]	6.7[a]	5.5[b]
Biceps femoris	7.2[a]	6.4[b]	5.6[c]	5.0[c]	7.0[a]	6.4[a]	5.7[b]	5.2[b]
Semimembranosus	6.1[a]	5.3[b]	5.3[b]	4.6[c]	5.4[ab]	5.7[a]	5.4[ab]	4.8[b]
Overall tenderness								
Longissimus	6.3[a]	6.1[a]	6.2[a]	4.6[b]	6.4[a]	6.1[ab]	5.8[ab]	4.9[b]
Biceps femoris	6.7[a]	6.1[a]	5.0[b]	4.8[b]	6.6[a]	6.0[a]	5.0[b]	4.9[b]
Semimembranosus	5.7[a]	5.1[ab]	5.0[b]	4.0[c]	5.6[a]	5.4[a]	4.6[b]	4.2[b]
Connective tissue tenderness								
Longissimus	6.8[a]	6.7[a]	6.5[a]	5.6[b]	6.9[a]	6.7[a]	6.4[a]	5.5[b]
Biceps femoris	6.6[a]	6.1[a]	5.3[b]	5.2[b]	6.6[a]	6.1[ab]	5.4[b]	5.0[b]
Semimembranosus	6.2[a]	5.4[b]	5.3[b]	4.7[b]	5.9[a]	5.8[a]	5.2[b]	4.7[b]
Shear force value, kg								
Longissimus	4.4[a]	6.0[b]	6.3[b]	7.9[c]	4.9[a]	5.6[ab]	6.4[b]	7.7[c]
Biceps femoris	4.5[a]	5.5[bc]	5.0[ab]	5.9[c]	5.1[a]	5.1[a]	4.7[a]	6.3[b]
Semimembranosus	5.7[a]	6.7[b]	7.8[c]	7.8[c]	6.1[a]	7.0[b]	6.9[ab]	8.2[c]
Sarcomere length, μm								
Longissimus	1.77[ab]	1.78[a]	1.78[a]	1.72[b]	1.79[a]	1.79[a]	1.76[ab]	1.73[b]
Biceps femoris	1.84[ab]	1.82[ab]	1.85[a]	1.79[b]	1.85[a]	1.86[a]	1.79[b]	1.81[ab]

[a,b,c] Means in the same horizontal row and within the same group (fat thickness or marbling) bearing different superscripts are different ($P < 0.05$).

[d] The population of carcasses (n = 40) was divided into quartiles (n = 10) according to thickness of subcutaneous fat opposite the longissimus muscle at the 12th rib. The means, maximum and minimum fat thickness for each quartile are as follows: quartile I = 7.6, 10.2 and 5.2 mm; quartile II = 4.0, 5.1 and 3.0 mm; quartile III = 2.3, 3.0 and 1.8 mm; quartile IV = 1.0, 1.5 and 0.7 mm.

[e] The population of carcasses (n = 40) was divided into quartiles (n = 10) according to the USDA marbling score in the longissimus muscle at the 12–13th rib interface. The means, maximum and minimum marbling scores for each quartile are as follows: quartile I = 22, 29 and 19; quartile II = 16, 18 and 13; quartile III = 12, 13 and 11; quartile IV = 9, 11 and 6 (where 30 is abundant-plus, 29 is abundant-typical, etc.).

gested that increased postmortem muscle temperature (which would occur in fatter carcasses, Table 2) would enhance the activity of muscle cathepsins under conditions of lower pH (Table 3) and higher temperature (Table 2). Dutson et al. (1975) reported that tenderness increases via delayed chilling result from a combination of a reduction in cold shortening and an increase in autolytic enzyme activity. The latter researchers concluded that delayed chill and increased tenderness may normally occur as a result of increased carcass mass and/or fatness (Dutson et al., 1975). The largest increases in tenderness in the present study were observed at fat thicknesses opposite the longissimus muscle of >1.8 mm (longissimus), >3.0 mm (biceps femoris) and >5.2 mm (semimembranosus).

Juiciness ratings, tenderness scores, shear force values and sarcomere lengths for three muscles from each lamb carcass in four marbling score groups are presented in Table 6. To more precisely identify the effects of intramuscular fat deposition on tenderness, the complete population (n = 40) of carcasses was divided into quartiles (n = 10) according to marbling score in the longissimus muscle at the 12–13th rib interface. Lambs in quartile I, (moderate-minus to abundant-typical amounts of marbling) were more tender ($P < 0.05$) than lambs in quartiles II, III and IV in 1 of 15, 7 of 15 and 14 of 15 comparisons, respectively. Lambs in quartile II (small-minus to modest-plus amounts of marbling) were more tender ($P < 0.05$) than lambs in quartiles III and IV in 5 of 15 and 13 of 15 comparisons, respectively. Lambs in quartile III (slight-typical to small-minus amounts of marbling) were more tender ($P < 0.05$) than lambs in quartile IV (practically devoid-plus to slight-minus amounts of marbling) in 6 of 15 comparisons. The data suggest that increased deposition of intramuscular fat in the longissimus muscle at the 12–13th rib interface is associated with increased tenderness of the longissimus, biceps femoris and semimembranosus muscles, but that the relationship is not linear. McBee and Wiles (1967) and Smith et al. (1969) reported that the tenderness of beef loin and rib steaks increased with additional degrees of marbling, but that there were not always significant differences with each successive increase in degree of marbling.

The data of this study (Table 6) reveal significant decreases in tenderness between adjacent marbling score quartiles in 1, 5 and 6 of 15 comparisons between quartiles I and II, II and III and III and IV, respectively. Sarcomere length did not differ between quartiles I and II or III and IV for either muscle (Table 6). The effect of marbling on lamb tenderness does not appear to be exerted through its influence (via changes in cold shortening effects) on sarcomere length. The largest increases in tenderness were observed at marbling scores in the longissimus muscle at the 12–13th rib interface of > slight-minus (longissimus), > small-minus (biceps femoris) and > slight-minus (semimembranosus).

Analyses of variance of data in Table 6 revealed significant ($P < 0.05$) interactions between subcutaneous fat thickness and marbling score for 2 of the 5 sensory panel ratings. Correspondingly, juiciness ratings, tenderness scores, shear force values and sarcomere lengths were analyzed by subcutaneous fat thickness × marbling score groups and results are presented in Table 7. In subcutaneous fat quartile I, higher marbling scores (> moderate-typical) were associated with lower ($P < 0.05$) scores for tooth pressure and fragmentation, but marbling was not generally related to tenderness, juiciness or sarcomere length among carcasses with 5.2–10.2 mm of external fat. Similar results were noted for lambs in quartiles II and III with regard to tenderness and juiciness, but sarcomeres were shorter for lambs with lower marbling scores in 3 of 4 comparisons. In quartile IV (lambs with 0.7–1.5 mm of subcutaneous fat thickness), higher marbling scores (> slight-minus) were associated ($P < 0.05$) with higher tenderness ratings (in 5 of 12 comparisons) and lower shear force values (in 3 of 3 comparisons).

Data in Table 7 suggest that increased marbling is of greatest importance in determining tenderness when subcutaneous fat is not present in adequate quantities. Moreover, marbling seems to be more critical in determining tenderness of the longissimus muscle than it is for the more massive biceps femoris and semimembranosus muscles. McCrae et al. (1971) demonstrated that postmortem shortening of myofibrils decreases the tenderness of lamb muscles. Postmortem shortening is dependent on temperature, but not all muscles show the same temperature dependence (Newbold and Harris, 1972). McCrae et al. (1971) found that the longissimus and semimembranosus muscles were toughened considerably by early freezing while the biceps femoris muscle was less affected by cold shock. The latter researchers concluded that there was no intrinsic difference among these muscles in their potential to shorten, rather the difference lies in the degree of stretch or slack imposed on them by their skeletal attachments (McCrae et al., 1971). It does not appear likely that marbling influences tenderness by decreasing the extent of myofibrillar cold shortening since there was no difference in sarcomere length between marbling score groups within subcutaneous fat thickness quartile IV (Table 7).

The most salient evidence for the proposed relationship between subcutaneous fat, rate of chilling and tenderness of lamb is provided in Table 8. These data characterize differences between the left and right sides of lamb carcasses in the intermediate finish group. Significant differences between trimmed and untrimmed sides were observed for internal temperature of the longissimus muscle during chilling (at 1, 2 and 3 hr postmortem), sarcomere length of longissimus and biceps femoris muscles, and 19 of 34 measures of tenderness for longissimus, biceps femoris and semimembranosus muscles. Differences in tenderness between sides were most evident for the longissimus muscle and least pronounced for the biceps femoris muscle. The longissimus muscle is relatively narrow and thin, is not located in the proximity of other muscles of substantive thickness and could sustain shortening of sarcomeres in response to the cold and/or could sustain a decrease in the extent of autolytic enzyme activity if it is not adequately insulated by fat around its external surfaces.

Smith (1968) studied 39 carcass traits and reported that lower flank streaking score and percentage of kidney and pelvic fat were most closely associated with tenderness of the lamb longissimus muscle. It appears that kidney fat would insulate the longissimus muscle from the interior during chilling thereby decreasing its chilling rate and increasing its ultimate tenderness. The tenderness of the biceps femoris muscle was not affected by the presence or absence of subcutaneous fat which indicates that this muscle is sufficiently insulated from cold shock by its own bulk, by its location adjacent to other thick muscles in the hind leg or by intramuscular and/or intermuscular fat deposition. Since the tenderness of the semimembranosus muscle was affected by the presence of subcutaneous fat, an alternative explanation is needed. Smith et al. (1969) found a tenderness gradient within the beef longissimus muscle which can be explained by consideration of the differential rate of chilling which occurs across the cross-section of that muscle (toughness was greatest in that section of the longissimus which was least protected from cold shock). It is possible that a similar cross-sectional gradient in tenderness occurs in the biceps femoris muscle and that the samples evaluated by the sensory panel were from areas which are not affected as extensively by cold shock as are areas near the periphery. It is also possible that (as previously noted) the findings of McCrae et al. (1971) apply, in that the biceps femoris is prevented from shortening by the stretch placed upon it by its skeletal attachments. On the other hand, rate of chilling cannot be the complete answer in explaining observed

differences in tenderness; otherwise, thick muscles in thick cuts would be the most tender. The fact that large quantities of connective tissue occur in certain muscles of the hind leg (Wenham et al., 1973; Cross et al., 1972) most assuredly plays a role in determining the tenderness of muscles like the biceps femoris.

In data not presented in tabular form, palatability ratings, shear force values and sarcomere lengths from the longissimus muscle of trimmed sides of lambs with intermediate finish were divided into two groups based on differences in marbling score. A comparison of lambs with small-typical or higher marbling scores (group I) with lambs having small-minus or lower marbling scores (group II) generally supported relationships discussed earlier (Tables 6 and 7). Longissimus muscles from the trimmed sides of carcasses in group I had mean sarcomere lengths, shear force values and overall tenderness ratings of 1.75 μm, 6.0 kg and 5.6; corresponding means for longissimus muscles from lambs in group II were 1.71 μm, 7.6 kg and 4.5, respectively. The four carcasses in the intermediate group which had the lowest marbling scores (slight-typical) had a mean sarcomere length of 1.71 μm a mean shear force value of

Table 7—Juiciness ratings, tenderness scores, shear force values and sarcomere lengths for three muscles from lamb carcasses in each subcutaneous fat thickness group stratified according to marbling scores

	Subcutaneous fat thickness[c]							
	Quartile I		Quartile II		Quartile III		Quartile IV	
Trait	Higher marbling scores[d]	Lower marbling scores[e]	Higher marbling scores[d]	Lower marbling scores[e]	Higher marbling scores[d]	Lower marbling scores[e]	Higher marbling scores[d]	Lower marbling scores[e]
Juiciness								
Longissimus	6.0	6.2	6.4	6.0	6.2	6.0	5.7	6.2
Biceps femoris	5.6	5.5	5.5	5.4	4.9	5.4	4.7	5.0
Semimembranosus	5.0	5.9	5.1	5.2	5.4	5.1	4.7	4.8
Tooth pressure								
Longissimus	7.0	7.2	6.7	6.4	6.7	6.2	5.8[a]	4.5[b]
Biceps femoris	7.0	6.9	6.6	6.2	4.9	5.8	5.2	4.4
Semimembranosus	5.4[b]	6.5[a]	4.8	5.0	5.1	5.3	4.8	3.9
Fragmentation								
Longissimus	7.3	7.3	6.9	6.7	7.0	6.7	6.2[a]	4.8[b]
Biceps femoris	7.2	7.3	6.7	6.1	5.1[b]	6.1[a]	5.4	4.5
Semimembranosus	5.4[b]	6.7[a]	5.2	5.5	5.0	5.6	5.1[a]	4.1[b]
Overall tenderness								
Longissimus	6.3	6.3	6.0	6.2	6.1	6.3	5.3[a]	4.0[b]
Biceps femoris	6.7	6.7	6.1	6.0	4.6	5.5	5.3	4.4
Semimembranosus	5.7	5.7	5.0	5.3	5.0	4.9	4.2	4.0
Connective tissue tenderness								
Longissimus	7.0	6.7	6.5	6.9	6.7	6.3	6.1[a]	5.0[b]
Biceps femoris	6.7	6.5	6.2	5.9	4.9	5.7	5.6	4.8
Semimembranosus	6.1	6.3	5.4	5.3	5.6	5.0	5.1	4.3
Shear force value, kg								
Longissimus	4.6	4.2	6.1	6.0	5.5[a]	6.9[b]	6.9[a]	8.9[b]
Biceps femoris	4.5	4.6	5.0	6.0	5.3	4.9	4.8[a]	7.0[b]
Semimembranosus	5.9	5.5	6.8	6.6	7.9	7.8	6.6[a]	9.0[b]
Sarcomere length, μm								
Longissimus	1.78	1.76	1.82[a]	1.74[b]	1.82[a]	1.74[b]	1.74	1.75
Biceps femoris	1.84	1.84	1.90[a]	1.74[b]	1.86	1.83	1.80	1.79

[a,b] Means in the same horizontal row and within the same fat thickness quartile bearing different superscripts are different ($P < 0.05$). Means underscored by a common line are not significantly different.

[c] Subcutaneous fat thickness quartiles are described in footnote d of Table 6.

[d] Higher marbling scores = the 5 carcasses within a subcutaneous fat thickness quartile which had the highest marbling scores in the longissimus muscle at the 12–13th rib interface. The means, maximum and minimum marbling scores for each quartile are as follows: quartile I = 23, 26 and 21; quartile II = 21, 29 and 18; quartile III = 14, 18 and 12; quartile IV = 12, 12 and 11 (where 30 is abundant-plus, 29 is abundant-typical, etc.).

[e] Lower marbling scores = the 5 carcasses within a subcutaneous fat thickness quartile which had the lowest marbling scores in the longissimus muscle at the 12–13th rib interface. The means, maximum and minimum marbling scores for each quartile are as follows: quartile I = 15, 19 and 12; quartile II = 14, 18 and 8; quartile III = 11, 12 and 11; quartile IV = 8, 11 and 6 (where 30 is abundant-plus, 29 in abundant-typical, etc.).

8.0 kg and a mean overall tenderness rating of 4.0. These results substantiate the conclusion that marbling is most effective in determining tenderness when the subcutaneous fat covering is very thin.

In conclusion, the data of the present study reveal that lamb carcasses or sides which have increased quantities of fat (subjectively determined via live evaluations of finish or objectively determined via fat thickness measurements and marbling scores): (a) chill more slowly, Tables 2 and 8; (b) maintain muscle temperatures conducive to autolytic enzyme degradation for greater periods of time postmortem, Tables 2 and 8; (c) sustain less shortening of sarcomeres, Tables 3, 6, 7 and 8; (d) have muscles with lower ultimate pH values, Table 3; (e) have less perceptible or softer connective tissue, Tables 4, 5, 6, 7 and 8; and (f) are more tender, Tables 4, 5, 6, 7 and 8 than lamb carcasses or sides which have limited quantities of fat. The data support the hypothesis that deposition of increased quantities of subcutaneous or intramuscular fat (particularly in carcasses with limited quantities of subcutaneous finish) increases tenderness via changes in postmortem chilling rate. An increased quantity of fat decreases the rate of temperature decline (either by insulation or via an increase in carcass mass), enhances the activity (or increases the duration of active proteolysis) of autolytic enzymes in muscle, lessens the extent of myofibrillar shortening and thereby increases the ultimate tenderness of cooked meat from a fatter carcass.

Table 8—Muscle temperatures, histological and chemical traits, tenderness ratings, shear force values and cooking data for three muscles from lambs in the intermediate finish group

	Longissimus (L) Int. finish group		Biceps femoris (BF) Int. finish group		Semimembranosus (SM) Int. finish group	
Trait	Untrimmed[c]	Trimmed[d]	Untrimmed[c]	Trimmed[d]	Untrimmed[c]	Trimmed[d]
Temperature (° C)						
0.0 hr postmortem	26.3	26.6	–	–	–	–
0.5 hr postmortem	19.8	18.4	–	–	–	–
1.0 hr postmortem	15.6[a]	13.4[b]	–	–	–	–
2.0 hr postmortem	10.8[a]	8.3[b]	–	–	–	–
3.0 hr postmortem	7.3[a]	5.7[b]	–	–	–	–
4.0 hr postmortem	3.8	3.3	–	–	–	–
5.0 hr postmortem	2.5	1.7	–	–	–	–
6.0 hr postmortem	0.9	0.2	–	–	–	–
Histochemical traits						
Sarcomere length (μm)	1.78[a]	1.73[b]	1.83[a]	1.77[b]	–	–
pH value	5.70	5.69	5.71	5.76	–	–
Tenderness profile						
Juiciness	6.1	6.0	5.1	5.0	5.3	5.1
Tongue-cheek pressure	7.1	7.0	6.3	6.3	6.1	6.0
Tooth pressure	6.5[a]	6.1[b]	5.7[a]	5.3[b]	5.2[a]	4.6[b]
Mealiness	3.4[a]	3.1[b]	3.2	3.0	2.8	2.6
Adhesion	6.8[a]	6.4[b]	5.9	5.6	5.5[a]	4.8[b]
Fragmentation	6.8[a]	6.3[b]	5.8	5.5	5.5[a]	4.9[b]
Connective tissue amount	8.3	8.1	8.0	7.8	7.7[a]	7.3[b]
Connective tissue softness	8.4	8.3	8.1	7.9	7.6[a]	7.2[b]
Tenderness rating						
Muscle fiber	6.6[a]	5.4[b]	6.2	5.9	5.8[a]	5.2[b]
Connective tissue	6.6[a]	5.8[b]	5.6	5.7	5.6[a]	5.1[b]
Overall	6.0[a]	5.0[b]	5.6	5.3	5.2[a]	4.6[b]
Shear force value						
Leg (kg)	–	–	5.0[a]	5.8[b]	7.2	7.8
Loin (kg)	6.1[a]	6.8[b]	–	–	–	–
Rib (kg)	8.6[a]	9.6[b]	–	–	–	–
Cooking data						
Weight loss (%)	19.6	17.9	–	–	–	–
Cooking time (min)	29.1	29.8	–	–	–	–

[a,b] Means in the same horizontal row and for the same muscle bearing different superscripts are different ($P < 0.05$).

[c] The right side of each lamb in the intermediate finish group was trimmed to remove all subcutaneous fat from the rack, loin, sirloin and leg cushion areas. Fat was removed from the dorsal midline to a point approximately 10 cm ventral to the midline (rack-sirloin) and completely around the leg.

[d] The left side of each lamb in the intermediate finish group was not trimmed, all subcutaneous fat and the fell membrane were left on this side of the carcass.

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Ms received 10/24/75; revised 1/19/76; accepted 1/26/76.

T.A. 12205, Texas Agricultural Experiment Station. This study was partially supported by the Natural Fibers and Food Protein Committee of Texas, Austin, TX and by a grant from King Ranch, Inc., Kingsville, TX.

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MEAT WITH HIGH LINOLEIC ACID CONTENT: OXIDATIVE CHANGES DURING FROZEN STORAGE

ABSTRACT

"High linoleic" meat with fat containing up to 20% linoleic acid was produced by feeding a protected lipid supplement to sheep and steers. A comparison was made of the frozen storage life of this meat and of conventional meat, packaged in sealed polyethylene film pouches and stored at −10°C or at −20°C. Peroxide development was much more rapid in adipose tissue from high linoleic meat stored at −10°C. Rate of peroxide development in high linoleic meat was greatly decreased when stored at −20°C. Taste panel assessments indicated that high linoleic meat stored at −10°C developed rancid odors and flavors 2–3 times more rapidly than did conventional meat.

INTRODUCTION

WHEN MEATS are stored at temperatures of −7°C or lower, microbial growth no longer occurs and storage life is then determined by the effects of chemical or biochemical changes. In the presence of oxygen, oxidative reactions are usually of greatest importance and storage life is then limited by the development of oxidative rancidity in fat (Hiner et al., 1951). The fat from ruminant animals is usually more saturated than that from nonruminants and stability with respect to oxidative changes decreases with increase in unsaturation of lipids. This is likely to be an important factor in the generally greater stability with respect to oxidative changes of the meat from ruminants compared to that from nonruminants.

However, Scott et al. (1970, 1971, 1972) and Cook et al. (1970) have recently shown that in ruminants it is possible to produce milk and body fat with a nearly tenfold increase in linoleic acid over normal concentrations. By feeding the animals a diet containing a polyunsaturated oil, protected from hydrogenation in the rumen by a formaldehyde-treated protein, the unsaturated fatty acids are incorporated into the tissues and milk fat.

It was expected that under aerobic conditions the meat from animals fed this special supplement, which will afterwards be referred to as high linoleic meat, would have a shorter life in frozen storage than meat from conventionally fed ruminant animals. However, this may not be so if the content of antioxidants in the meat were also increased as a result of the treatment. Therefore, as part of a program investigating the changes in meat properties brought about by feeding animals protected oil supplements, a comparison was made of the frozen storage life of high linoleic and conventional meat. The results are reported here.

EXPERIMENTAL

ONLY a limited amount of experimental material from animals fed the special supplement was available. For these first exploratory investigations sampling at frequent occasions over a prolonged period was favored to infrequent sampling with replication. This restricted the statistical analysis that could be carried out on the data.

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Preparation of samples

Details of the animals used and their diets for the last weeks before slaughter, are given in Table 1. Following slaughter, the carcasses from the animals were placed in a chiller with the temperature controlled to 1°C ± 0.5°C.

Except for samples in experiment 8 (Table 1), samples for storage experiments were packaged and placed in frozen storage the day following slaughter of the animal. In experiment 8, the primal cuts required were removed from the carcass the day following slaughter, but packaging of samples was delayed for 3 days; the meat was first held for 1–2 days in a room at 7°C and subsequently in a room at 1°C.

Taste panel assessments were carried out on the muscles and fat removed from along the back bone (major muscle the longissimus dorsi). This strip of meat was cut as uniformly as possible into slices 1.9 cm thick which were packaged individually. 2-Thiobarbituric acid (TBA) values were determined on muscle portions removed from these samples. Peroxide values were determined on samples of adipose tissue trimmed from the carcass and stored in 50-g portions.

Samples to be stored aerobically were sealed in tight fitting pouches made from low density polyethylene film 0.038 mm thick of similar quality to that used by Ledward and Macfarlane (1971). This material is permeable to oxygen but provides a good water vapor barrier. In addition these samples were stored in jacketed freezers (Bate, 1968) which would further reduce the possibility of moisture loss during storage.

Samples to be stored under anaerobic conditions were sealed in evacuated pouches made from laminated flexible material of high quality, composed of 50 gauge mylar film bound by polyethylene 0.05-mm thick to 0.635 mm aluminum foil which had a 0.05-mm polyethylene coating on the inside.

Analytical techniques

Linoleic acid content of adipose tissue was estimated by preparing methyl esters which were examined by means of gas chromatography, in the manner described by Ford et al. (1975).

As required for analysis, samples were removed from frozen storage and held overnight to thaw at 5°C.

Peroxide and free fatty acid values of adipose tissue were determined according to the methods described by Rockwood et al. (1947).

2-Thiobarbituric acid (TBA) values were determined on muscle using a distillation technique (Tarladgis et al., 1960) except that the TBA reagent-distillate mixture was held at ambient temperature for 16 hours to allow color development as described by Tarladgis et al. (1964) (K-value 7.85).

Taste evaluations were carried out by a panel of 18 trained tasters with some experience in evaluating high linoleic meats. Each panel member was served a sample of muscle with attached fat. The amount of fat available varied greatly according to the fatness of the animals.

Meat samples for taste assessment were cooked for 15 min in a forced draft convection oven at 230°C. They were then held in covered dishes in an oven at 80°C for at least 10 min before they were served to panellists. Some tests were carried out as paired comparisons in which panellists were asked to assess the difference, if any, in rancid odor and flavor between aerobically or anaerobically (−40°C) stored samples of both high linoleic and conventional meat.

Other taste evaluations were carried out in which panellists were requested to assess samples for (i) the intensity of a characteristic aroma and flavor associated with high linoleic meat, referred to at this Laboratory as "poly" aroma or flavor, (ii) the intensity of rancid odor and flavor and (iii) acceptability. For the assessments of aroma and flavor, a 9-point structured scale was employed, ranging from none to very strong. For acceptability a hedonic scale ranging from very good to very poor was employed.

Table 1—Details of experimental animals

Exp No.	Animal species and breed	Number of animals: Basal diet[a]	Number of animals: Supplemented diet[b]	Duration of special feeding (days)	Mean linoleic acid content of fatty tissue[c] %	
1	Border Leicester	10		39	1.62	(0.33)
	X Merino lambs		10	39	19.1	(1.48)
2	Border Leicester	10		55	2.5	(—)
	X Merino yearling sheep		10	55	14.9	(—)
3	Border Leicester	3		42	1.43	(0.23)
	X Merino lamb ewes		3	14	9.2	(0.6)
			3	28	19.0	(3.1)
			3	42	21.6	(0.93)
4	Border Leicester	—	3	35	11.6	(1.86)
	X Merino lamb wethers		3	42	19.5	(1.49)
5	Merino wether lambs	8		42	2.43	(0.34)
			8	42	20.51	(0.81)
6	Mature Merino wethers	6		42	2.8	(—)
			6	21	8.4	(1.5)
			6	42	15.4	(1.20)
7	Mature Merino ewes	10		70	—	
			15	70	15.5	(0.82)
8	Angus yearling steers	4		68	1.35	(0.18)
			4	68	19.1	(1.39)

[a] The composition of the basal diet was chopped alfalfa and oats, mixed in equal proportions by weight.
[b] The composition of the supplemented diet was chopped alfalfa, oats and protected sunflower seed-casein supplement (50:20:30, Scott et al., 1972).
[c] % of total fatty acids in subcutaneous fat samples (Ford et al., 1975). The figure in brackets is the standard error of the mean.

RESULTS

Peroxide value

The results for experiments 1 and 2 (Table 1) are presented in Figure 1. Peroxide development was found to be much more rapid in adipose tissue from high linoleic meat than in that from conventional meat. Peroxide development was at about the same low rate in the adipose tissue samples from meat of both conventionally fed lamb and yearling sheep. However, peroxide development was markedly faster in samples from high linoleic sheep meat than in those from high linoleic lamb meat.

In Figures 2 and 3 the results are presented for the experiments 3 and 6 respectively, Table 1. They show the effect on peroxide development of varying the duration of feeding the special supplement. The results for −10°C storage presented in Figure 2 indicate that there was no apparent difference between the stability of the 2- and 4-wk treatments. Also, these treatments were only slightly more stable than the 6-wk treatment. In the results presented in Figure 3, the samples from animals fed supplement for 3 wk were markedly less stable than those from conventional meat, and in this case there appeared to be a progressive decrease in stability in the meat from animals fed supplement for nil, 3 and 6 wk. However, even in the samples from animals fed the special supplement for 6 wk, there was no marked indication of an accelerating rate of peroxide development in the second half of the storage period as there was in the results presented in Figures 1 and 2.

Data for the rate of peroxide development in the adipose tissue from high linoleic beef animals (experiment 8, Table 1) stored at −10°C are included in Figure 4. From comparisons of the data in this figure with data in Figures 1, 2 and 3, it could be deduced that the samples from high linoleic beef deteriorated more rapidly than those from high linoleic sheep or lamb. However, this accelerated deterioration may, at least in part, be a consequence of the delay in packing and freezing the beef samples mentioned in the experimental section.

Inspection of Figures 2 and 4 shows that decreasing the storage temperature from −10°C to −20°C resulted in a large decrease in the rate of peroxide development. Adipose tissue from experiments 4 and 5 (Table 1), was also stored at −20°C. After 6 and 12 months' storage, peroxide values for the high linoleic samples from experiment 4 were 1.39 ± 0.9 and 1.85 ± 0.8 meq/kg respectively; only traces of peroxide were observed in the samples from experiment 5 after 12 months' storage. No peroxide was detected in samples stored anaerobically for up to 12 months.

Free fatty acid values

Free fatty acid content was also estimated on the fat samples used to obtain the data on peroxide values, as reported in Figures 1 to 4 inclusive. In all cases this remained low (range 0.2–0.8% free fatty acid as oleic acid), with perhaps only a slight increase over the duration of the storage experiments. No differences were apparent between the samples of conventional and high linoleic fat.

TBA values

At about the times that adipose tissue samples were taken to obtain the results reported in Figures 1 to 4, muscle samples were taken for the determination of TBA values. Except in some muscle samples from the sheep of experiment 2 (Table 1), no differences were apparent in TBA values between stored conventional and high linoleic meat.

Taste panel assessment

In the paired comparison tests on samples from experiments 1, 2, 6 and 8, samples were evaluated for the first time 30 days after preparation, then regularly at intervals of 2–3 wk for approx 1 yr. In all tasting sessions more panel members consistently identified rancid odor and flavor in the aerobic samples of high linoleic meat than in the samples of conventional meat. The storage times after which 50% or more of taste-panel members consistently identified rancidity in a meat sample stored aerobically at −10°C when compared to a sam-

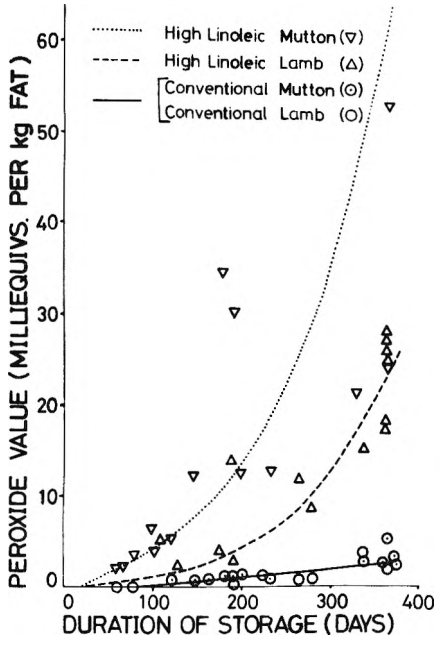

Fig. 1—Peroxide development in adipose tissue from conventional and high linoleic sheep carcasses stored aerobically at −10°C (experiments 1 and 2, Table 1).

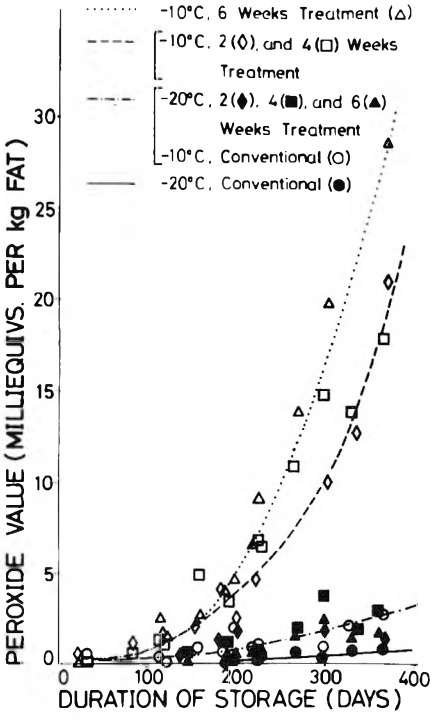

Fig. 2—Peroxide development in adipose tissue stored aerobically at −10°C or −20°C from sheep fed a protected lipid diet for 0, 2, 4 or 6 wk (experiment 3, Table 1).

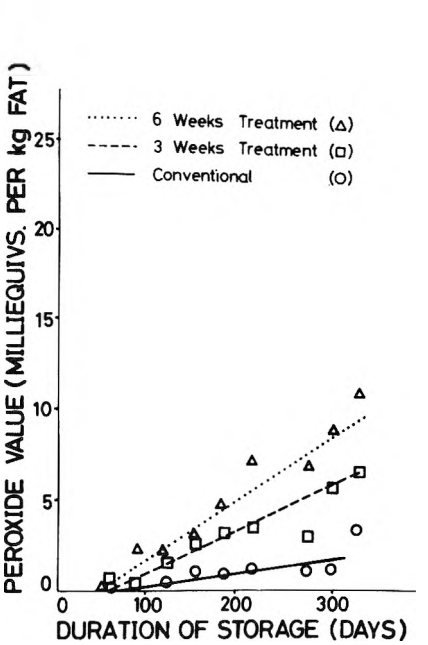

Fig. 3—Peroxide development in adipose tissue stored aerobically at −10°C from sheep fed a protected lipid diet for 0, 3 or 6 wk (experiment 6, Table 1).

ple of the same meat stored anaerobically at −40°C are listed in Table 2. These results suggest that high linoleic meat stored aerobically at −10°C developed rancid odors and flavors 2–3 times more rapidly than did conventional meat.

Table 3 presents the results of a taste-panel evaluation of various attributes of mutton samples (experiment 7, Table 1) over 3 wk of storage at −10°C. These results show that the intensity of the "poly" aroma and flavor of high linoleic meat decreased significantly during frozen storage. Rancid odor and flavor were scored significantly higher in the high linoleic than in the corresponding conventional meat samples. The scores increased significantly over the duration of the experiment for the high linoleic samples but not for the conventional meat samples. These changes had a small but significant effect on the acceptability of the samples. The amount of mutton available was insufficient to allow panel members a preliminary tasting session to re-introduce them to "poly" flavor. This could be the cause of the small positive values obtained for "poly" aroma and flavor of conventional meat at zero storage time. Analysis of variance showed a significant difference between panel scores for high linoleic and conventional meats in all attributes.

Table 2—Storage time after which 50% or more of taste-panel members consistently identified a meat sample stored aerobically at −10°C when compared with a sample of similar meat stored anaerobically at −40°C[a]

Meat type	Source of meat exp no. from Table 1	Approx storage time (days) for 50% of panel members to detect difference	
		Rancid odor	Rancid flavor
Conventional lamb	1	200	200
High linoleic lamb	1	85	110
Conventional sheep meat	2	150	150
High linoleic sheep meat	2	70	45
Conventional sheep meat	6	120	150
High linoleic sheep meat	6 (21 days supp)	85	70
	6 (42 days supp)	85	56
Conventional beef	8	325	230
High linoleic beef	8	150	100

[a] Panellists were asked to identify which, if any, of the samples had a stronger rancid odor or flavor.

At the beginning of the storage period peroxide values in both the high linoleic and the control samples were too small to be measured. After 3 wk storage the mean peroxide values were similar, at 0.22 and 0.21 meq/kg respectively.

Table 4 presents the results of a taste-panel assessment of meat stored at −20°C for up to 1 yr. It can be seen that the differences between conventional and high linoleic meat were not significant at 6 months' storage, but were so at 9 and 12 months' storage. Table 5 presents the results of a taste-panel evaluation of high linoleic meat from the animals of experiment 4 (Table 1) at 6 and 12 months' storage. In this experiment, the panel did not have conventional meat with which to compare the high linoleic meat. The results of the panel assessment were very similar at both storage times.

DISCUSSION

THE MAIN PURPOSE of this investigation was to gauge the stability during frozen storage under aerobic conditions of

Table 3—Mean taste-panel scores for intensity of "poly" aroma and flavor, rancid odor and flavor, and for acceptability of high linoleic and of conventional mutton stored aerobically at −10°C for up to 3 wk

Duration of storage (wk)	"Poly"[a] aroma		Rancid[a] odor		"Poly"[a] flavor		Rancid[a] flavor		Acceptability[b]	
	C[c]	HL[d]	C	HL	C	HL	C	HL	C	HL
0	0.66	1.80	0.26	0.58	0.56	1.68	0.37	0.69	4.84	3.93
1	0.27	1.34	0.25	0.48	0.28	1.30	0.27	0.59	5.01	4.11
2	0.27	1.38	0.31	0.97	0.39	1.44	0.45	0.88	4.90	3.39
3	0.29	0.98	0.34	0.92	0.31	1.02	0.61	1.08	4.44	3.68
LSD[e]	0.24	0.42	0.20	0.44	0.30	0.33	0.27	0.32	0.43	0.47

[a] Intensity scores from 0, zero intensity, to 8, very strong intensity
[b] Hedonic scale, 0 — very poor acceptability, 8 — very good
[c] C: conventional meat
[d] HL: high linoleic meat
[e] Least significant difference between any two means, p = 0.05

Table 4—Mean taste-panel scores for rancid odor and flavor, and for acceptability of high linoleic and conventional lamb meat (experiment 5, Table 1) stored aerobically at −20°C.

Storage period (months)	Meat type	Rancid[a] odor	Rancid[a] flavor	Acceptability[b]
6	conventional	1.02	0.78	5.89
	high linoleic	1.11	1.22	4.86
9	conventional	0.39	0.38	5.86
	high linoleic	0.92*	1.03**	4.06***
12	conventional	0.54	0.65	5.24
	high linoleic	1.42**	1.75***	3.56**

[a] Intensity scores from 0, zero intensity, to 8, very strong intensity
[b] Hedonic scores from 0, very poor acceptability, to 8, very good
* Significant discrimination from basal diet at $P < 0.05$
** Significant discrimination from basal diet at $P < 0.01$
*** Significant discrimination from basal diet at $P < 0.001$

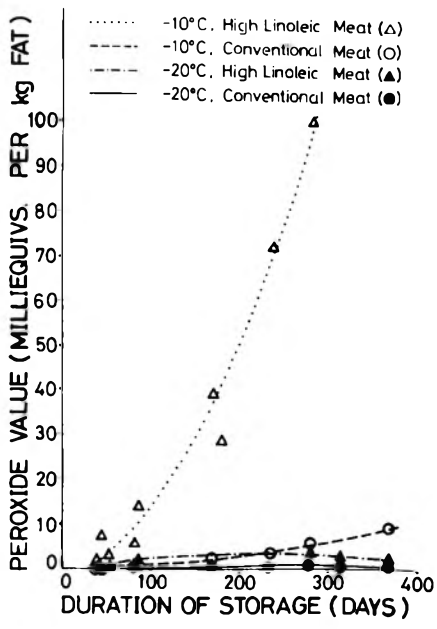

Fig. 4—Peroxide development in adipose tissue from conventional and high linoleic beef carcasses stored aerobically at −10°C or at −20°C (experiment 8, Table 1).

high linoleic sheep and beef meats compared with that of conventional meats. The investigations of changes in peroxide values during storage at −10°C, in fat samples with about 20% linoleic acid content, clearly showed that the stability of the adipose tissue was greatly reduced after linoleic acid content reached this level. Peroxide development in high linoleic samples was characterized by a short induction period, presumably corresponding to the destruction of natural antioxidants, followed by a rapidly increasing rate as in autoxidizing fats (Lundberg, 1962). In contrast, even towards the end of the 12 months' duration of the experiment, peroxide development in samples from conventional meat apparently had not passed the induction phase. Also, peroxide development did not relate well to the linoleic acid content of samples as given in Table 1 for although the results shown in Figure 3 indicate progressive instability with increasing linoleic acid content, those shown in Figure 2 reveal little differences in the relatively high rate of peroxide development regardless of whether samples contained about 9% or 19% linoleic acid. These apparent inconsistencies may be due to variations in the amount of antioxidants present. The changes in oxidative stability found here are in accord with the findings of Kimoto et al. (1974) on the effect of increasing the linoleic acid levels on the stability to autoxidation of rendered subcutaneous fats from steers. On the basis of our results a period of aerobic storage of even a few weeks at −10°C should be avoided for these meats. However, stability is greatly improved if storage is at −20°C, at which temperature the rate of peroxide development in the high linoleic adipose tissue samples approximated that in samples from conventional meat stored aerobically at −10°C.

Except for one experiment, there were comparable increases during storage in TBA values of both high linoleic and conventional meats. This was so even after prolonged storage, when the taste panel discriminated strongly against rancidity in the high linoleic samples. However, the TBA reaction is not a good indicator of oxidation of linoleate, particularly at the early stages (Dahle et al., 1962). It appears therefore that this reaction will be of little value for following oxidation of uncooked high linoleic meats.

Some of the difficulties in estimating the storage life of meat products by taste-panel assessment have been discussed by Jul (1969). He prefers to assess storage life in terms of the time taken to bring about a decrease in the organoleptic rating of 1 or 2 points using a 0–10 point scale. However the validity of this approach appeared to be in doubt in the present case because panel members had only recently been introduced to the new flavors of high linoleic meat. Their assessment of the acceptability of these meats might change as they became more accustomed to the flavor. Also, considerable variation

Table 5—Mean taste-panel scores for "poly" aroma and flavor, rancid odor and flavor, and acceptability of lamb meat (experiment 4, Table 1) stored aerobically at 20°C[a]

Storage period	Duration of feeding special supp	"Poly"[b] aroma	Rancid[b] odor	"Poly"[b] flavor	Rancid[b] flavor	Acceptability[c]
6 months	5 wk	2.17	1.15	2.24	1.10	4.23
	6 wk	2.24	1.43	2.65	1.34	3.92
12 months	5 wk	1.24	1.13	1.47	1.09	4.40
	6 wk	2.02	1.56	2.27	1.68	3.64

[a] Analysis of variance showed no significant difference between 5 wk and 6 wk feeding treatments or between rancidity scores at 6 months and 12 months storage.
[b] Intensity scores from 0, zero intensity, to 8, very strong intensity.
[c] Hedonic scores from 0, very poor acceptability, to 8, very good.

can occur between experiments in the flavor of high linoleic sheep meat (Park et al., 1976). Therefore the main approach adopted for the taste-panel assessment was to compare the test sample with another which had been stored under conditions where oxidative and other changes were expected to be negligible, i.e., anaerobically at a low temperature (−40°C). This procedure revealed that, as expected, panel members detected greater changes in aerobically stored high linoleic meats than in conventional meats. Although from these experiments it was not possible to compare rates of change precisely, the high linoleic meat stored at −10°C apparently deteriorated 2–3 times more rapidly than the conventional meat. Because no differences were apparent in free fatty acid development between these meats, dissimilarities between them in storage characteristics could not be attributed to differences in rates of lipolysis. The results presented in Table 3 indicate another factor that may influence the organoleptic acceptability of stored high linoleic meat, namely the apparent tendency for the intensity of "poly" flavor to decrease on storage. This may only be apparent, in that as rancid flavor increases, there could be a progressive masking of "poly" flavor.

The results of the comparison between high linoleic and conventional meats stored at −20°C presented in Table 4 indicate that the differences in development of rancidity and in acceptability between these meats increased markedly as the duration of storage was increased from 6 to 12 months. In this experiment the differences at 6 months' storage were not significant.

The taste-panel results, combined with the relatively slow rate of peroxide development at −20°C, indicate that the stability of these meats in the presence of oxygen is greatly increased as the temperature during storage is decreased. However, until wider experience of the storage of these meats at low temperatures is obtained, it would appear preferable to limit storage to about 3 months at −20°C to allow a margin for error. Where longer periods of storage are contemplated, oxygen should be excluded, for example, by vacuum packaging the product in a pouch made of oxygen-impermeable film.

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Ms received 10/9/75; revised 12/13/75; accepted 12/16/75.

This work was supported in part by the Australian Meat Research Committee and by Alta Lipids Australia Pty Limited.

The technical skills of Phillipa Hancock and the participation of staff as panel members is gratefully acknowledged.

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PROXIMATE ANALYSIS, FREE AMINO ACID, VITAMIN AND MINERAL CONTENT OF MICROWAVE COOKED MEAT

ABSTRACT

Beef, pork and lamb roasts were cooked by two 2450 MHz microwave ranges, one operated at 220V (1054W cooking power) and one at 115V (492W cooking power) and by a conventional gas oven (163 ± 3°C). The only significant effect related to power level of microwave ranges was retention of thiamine, riboflavin and niacin which was less in meat cooked by the microwave 115V than by the other two methods. There was a trend toward less retention of sodium, chloride, phosphorus and iron in meat cooked by microwaves than by the conventional method. Also, microwave cooking resulted in less formation of free amino acids than conventional cooking but total protein did not differ significantly.

INTRODUCTION

THE INCREASING nation-wide concern for nutrition cannot be directed realistically unless up-to-date information is available on the influence of new processing and preparation procedures on nutritive value and quality of foods. Watt and Murphy (1970) pointed out that food composition tables utilized by government agencies, food industries and research workers are inadequate in this respect. Because of its widespread use, Watt and Murphy included microwave heating among the processes on which research is needed. They also emphasized the lack of data on vitamin and mineral content of cooked meat representing all species.

Some research has been focused on vitamin retention, with thiamine receiving the greatest amount of attention. Consistent trends for retention of thiamine were not apparent among different species and cuts of meat cooked by microwaves (Kahn and Livingston, 1970; Kylen et al., 1964; Noble and Gomez, 1962; Thomas et al., 1949). However, in two investigations on riboflavin, there was less retention in meats cooked by microwaves than in those cooked by conventional methods regardless of species (Noble and Gomez, 1962; Thomas et al., 1949). The research of Thomas et al. (1949) was the only study found which cited data on niacin retention, and no marked influence of method of cookery was indicated.

Only the recent investigation of Ream et al. (1974) included observations on mineral content of microwave cooked meat. Their analyses included correlations between sensory attributes and mineral content, but did not determine the relationship between method of cooking and mineral retention.

This study was undertaken to determine trends in retention of selected vitamins and minerals in beef, pork and lamb cooked by microwaves. Since Gat'Ko (1965) suggested that meat cooked by high frequency retained more nitrogen than that cooked conventionally, analyses of both total protein and free amino acids were conducted. Moisture and fat content were determined not only to facilitate expression of other data on a dry, fat-free basis but to provide information for nutritionists concerning fat content of the three species of meat.

EXPERIMENTAL

Procurement and preparation of meat

The details of procuring, handling and cooking meat were reported in a previous paper (Korschgen et al., 1975). Longissimus muscle of beef and of pork and deboned leg of lamb (semimembranosus and biceps femoris muscles) were frozen (−34°C) and stored (−18 ± 3°C) until 24–26 hr prior to testing when they were tempered (3°C) in the package. End portions of each raw roast were removed for chemical analyses. One roast was obtained from each longissimus muscle of beef and of pork, and from each deboned leg of lamb. Weights of raw roasts after trimming were 1170 ± 194g, 903 ± 121g, and 1111 ± 73g, respectively, for beef, pork and lamb. For each species, 15 roasts were randomly assigned to cooking by microwave 220V (Litton Model 70/50, 2450 MHz, 220V, 11.5 amp., 1054W cookig power), microwave 115V (Amana Model RR-2, 2450 MHz, 115V, 14.5 amp., 492W cooking power) or conventional gas oven (163 ± 3°C). Microwave energy was applied intermittently with a 3-min cycling for the microwave range operated at 220V and 6-min cycling for the microwave range operated at 115V. All roasts were cooked uncovered and achieved a final internal temperature of 70°C at which they were cut for sensory evaluation (Korschgen et al., 1975).

Proximate analysis, free amino acid, vitamin and mineral contents

Total moisture, ether extractable fat, nitrogen and free amino acids were analyzed in the Agricultural Experiment Station Chemical Laboratories, University of Missouri-Columbia. Drippings were warmed to effect dispersion of the fat for a representative sample. Nitrogen was determined by macro-Kjeldahl, and free amino acids by amino acid analyzer. Prior to amino acid analysis, protein was precipitated by sulfosalicylic acid.

Thiamine, riboflavin and niacin contents of meat were determined by the chemical methods of the Association of Vitamin Chemists (1966). Thiamine was analyzed by the thiochrome method, riboflavin by the fluorometric method and niacin by the procedure based on the reaction of niacin with cyanogen bromide. Due to the limited quantity, it was not possible to conduct these analyses on the drippings obtained from the meat during cooking.

Iron, phosphorus, sodium and chloride determinations for meat and for drippings collected during cooking were made by personnel of the Trace Substance Center, University of Missouri-Columbia. Iron was determined by atomic absorption spectroscopy, phosphorus by a reduced heteropolymolybdate colorimetric method, sodium by flame photometer, and chloride by Cotlove (1963) coulometric-amperometric titration.

Statistical analyses

Experiments were replicated five times for each species of meat, and analysis of variance was applied to the data. Variation due to replication and/or animal was included as a source of variance in the statistical design. Duncan's (1955) new multiple range test was employed to locate significant differences among means for treatments. Where observations among treatments were not equal, the test for least significant difference was used (Snedecor and Cochran, 1967) to determine differences between means. Statistical significance of differences cited in this paper is $P < 0.05$.

RESULTS & DISCUSSION

Proximate analysis

Beef was the only species of meat where significant differences were found in proximate analysis of the roasts cooked by the three methods. Percent moisture in the microwave cooked beef was significantly less than that of beef cooked by the conventional method. This trend has been reported previously for beef roasts (Kylen et al., 1964). The protein content

of microwave cooked meat was greater than the conventionally cooked meat, but the difference was statistically significant only between beef cooked by the microwave 115V and the conventional methods (Table 1).

Percent moisture was greatest in the drippings from the three species of meat cooked by the microwave 115V method and was the least in the drippings from conventionally cooked roasts. Percent protein in the drippings was inversely related to moisture content (Table 1).

Percent fat was greater in the drippings from the pork cooked conventionally than in that from pork cooked by the microwave 115V method. However, fat content of the drippings from lamb roasts differed between the two microwave methods of cookery, and the greater amount was in the drippings of lamb cooked by the microwave 220V method. No explanation is apparent for this difference in trends between the two species of meat, since the percent fat in the raw roasts was similar (Table 1).

Free amino acid content

Free amino acid content tended to be greater in conventionally cooked meat than in that cooked by microwaves. Statistically significant differences were found for three amino acids in pork, for six in beef and for 13 in lamb. This trend for significantly greater amounts in the conventionally cooked meat occurred for valine and leucine in all three species of meat. Aside from these two, differences in free amino acid content varied with the species of meat (Tables 2, 3, 4).

Since data on free amino acid content were extremely limited for the drippings from beef, it seems unwise to make inferences. However, the drippings from conventionally cooked pork and lamb contained significantly greater amounts of valine, isoleucine, leucine and tyrosine than the drippings from microwave cooked meat (Tables 2, 3, 4).

Vitamin content

There was only one significant difference in vitamin content of the cooked meat. Thiamine content of beef cooked by the microwave 115V method was significantly less than that of the conventionally cooked product (Table 5).

On a percent retention basis, as shown in Table 5, thiamine was significantly higher in beef cooked by the microwave 220V and conventional methods than in beef cooked by the microwave 115V method. A similar trend was apparent for riboflavin and niacin retention in beef and for niacin in pork. However, in these instances, there was no statistically significant difference in retention between the two microwave methods. Since differences were not statistically significant between meat cooked by the microwave 220V and conventional methods, vitamin retention was not related solely to length of time exposed to high temperature (Table 5). Although power levels were not specified in all previous research, trends observed for vitamin retention were similar to those reported earlier (Kylen et al., 1964; Noble and Gomez, 1962; Thomas et al., 1949). However, thiamine retention in pork roasts was considerably higher for all three methods of cookery in this study than in the data reported by Kylen et al. (1964). Likewise, values for niacin retention were higher than those of Thomas et al. (1949).

Mineral content

There was only one significant difference in mineral content in meats cooked by the three methods. Sodium content of lamb cooked conventionally was significantly greater than the content of this mineral in lamb cooked by the two microwave methods (Table 6).

On a percent retention basis, there was a trend toward greater mineral retention in the conventionally cooked meat than in the microwave cooked product although not all of the differences were statistically significant. The significant differences were for phosphorus and iron retention in beef and for iron retention in lamb. The iron retention in the conventionally cooked beef was not significantly different from that cooked by the microwave 115V method (Table 6).

Mineral content of the drippings of the conventionally

Table 1—Mean percent moisture, fat and protein contents of roasts cooked by microwave and conventional methods

Species and constituent	Cooked meat[a]			Drippings[a]		
	Microwave		Conventional	Microwave		Conventional
	220V	115V		220V	115V	
Beef[b]						
Moisture	52.9 b	53.5 b	59.9 a	77.7 ab	81.8 a	72.3 b
Fat	15.1	14.7	11.3	4.7	3.5	3.4
Protein	30.8 ab	31.5 a	28.5 b	13.1 b	11.9 b	19.4 a
Pork[c]						
Moisture	62.1	60.4	62.1	81.4 a	83.9 a	69.3 b
Fat	4.8	5.3	4.0	0.8 ab	0.7 b	0.9 a
Protein	33.2	33.6	33.4	16.9 b	12.2 c	26.7 a
Lamb[d]						
Moisture	59.1	60.6	60.7	65.6 b	79.1 a	63.3 b
Fat	7.8	7.2	8.1	12.3 a	5.0 b	9.2 ab
Protein	32.3	31.5	27.3	16.8 b	12.1 c	21.4 a

[a] N = 5. Where letters differ within a constituent for cooked meat or for drippings within a species, means differ significantly ($P < 0.05$) from each other (Duncan, 1955).
[b] Raw beef: 67.7% moisture, 10.9% fat, 21.3% protein. N = 15.
[c] Raw pork: 72.5% moisture, 4.3% fat, 22.7% protein. N = 15.
[d] Raw lamb: 72.4% moisture, 5.7% fat, 21.1% protein. N = 15.

Table 2—Mean free amino acid content (mg/g, dry, fat-free basis) of beef roasts cooked by microwave and conventional methods

Free amino acid	Raw beef[a]	Cooked beef[b]			Drippings[c]		
		Microwave		Conventional	Microwave		Conventional
		220V	115V		220V	115V	
Taurine	0.76	0.56	0.50	0.62	2.74	3.05	2.64
Aspartic acid	0.36	0.08	0.08	0.11	0.58	0.81	0.88
Threonine	0.28	0.18	0.16	0.22	1.00	1.05	1.21
Serine	0.38	0.24	0.23	0.30	1.50	1.40	1.83
Glutamic acid	2.68	1.05	0.80	1.12	5.49	6.30	6.02
Proline	0.00	0.00	0.00	0.00	0.53	0.75	0.73
Glycine	0.45	0.30	0.28	0.32	1.86	2.03	1.74
Alanine	1.96	1.29	1.33	1.52	8.41	8.69	9.58
Valine	0.35	0.25 b	0.23 b	0.35 a	1.51	1.35	1.87
Methionine	0.22	0.13 b	0.12 b	0.19 a	0.70	0.68	0.84
Isoleucine	0.27	0.17 ab	0.16 b	0.23 a	1.00	0.94	1.16
Leucine	0.56	0.35 b	0.31 b	0.49 a	2.05	1.83	2.42
Tyrosine	0.32	0.19 b	0.16 b	0.25 a	1.11	1.03	1.31
β-Alanine	0.03	0.00	0.00	0.00	0.20	0.22	0.24
Phenylalanine	0.33	0.21 b	0.22 b	0.33 a	1.05	1.04	1.27
Ornithine	0.09	0.05	0.06	0.05	0.28	0.22	0.28
Lysine	0.42	0.26 ab	0.23 b	0.33 a	1.54	1.03	1.79
Histidine	0.21	0.13 b	0.12 b	0.17 a	0.71	0.70	0.88
Anserine	3.06	2.54	2.28	3.09	17.01	12.93	15.95
Carnosine	19.22	14.92	15.50	17.44	109.21	93.79	103.23
Arginine	0.50	0.34	0.28	0.34	2.21	1.89	2.53
Total	32.49	23.22 b	23.10 b	27.42 a	161.37	141.70	158.36

[a] N = 15.
[b] N = 5. Where letters differ for a free amino acid in cooked meat, means differ significantly ($P < 0.05$) from each other (Duncan, 1955).
[c] N = 2–4. No significant differences among means.

cooked meat was greater than that of the microwave cooked meat. A significant difference occurred for sodium, chloride, phosphorus and iron content of drippings from cooked pork. Also, this trend was apparent for phosphorus and iron content of drippings from beef and from lamb. Chloride content of drippings from cooked lamb was significantly different for all three methods with the least amount of this mineral in the drippings from lamb cooked by the microwave 115V method and the greatest amount in drippings from lamb cooked by the conventional method (Table 6). The greater concentration of minerals in the drippings from the conventionally cooked meat could be related to the lower moisture content of the drippings (Table 1).

Summary of trends

Analyses of the data revealed several general trends applicable to the three species of meat as follows:

1. Greater amounts of free amino acids were formed with conventional than with microwave cooking.
2. Retention of thiamine, riboflavin and niacin in meat was similar for the conventional and the microwave 220V methods, and both of these treatments were superior to the microwave 115V method for vitamin retention.
3. There was less retention of minerals, particularly phosphorus and iron, in microwave than in conventionally cooked beef.
4. On an edible basis, vitamin and mineral contents were similar in meats prepared by the three methods of cookery. The two exceptions to this were thiamine in beef and sodium in lamb.
5. Other than the effect on vitamins mentioned above, constit-

Table 3—Mean free amino acid content (mg/g, dry, fat-free basis) of pork roasts cooked by microwave and conventional methods

Free amino acid	Raw pork[a]	Cooked pork[b]			Drippings[c]		
		Microwave		Conventional	Microwave		Conventional
		220V	115 V		220V	115V	
Taurine	1.80	1.27	0.99	1.32	5.56	4.00	12.44
Aspartic acid	0.38	0.17	0.20	0.19	0.73	0.66	1.17
Threonine	0.25	0.15	0.15	0.19	0.85	0.67	1.96
Serine	0.29	0.19	0.16	0.22	1.02	0.78	1.98
Glutamic acid	1.54	0.78	0.79	0.85	4.52	3.72	5.09
Proline	0.00	0.00	0.00	0.00	0.58	0.51	1.76
Glycine	0.48	0.31	0.26	0.33	1.70	1.31	3.62
Alanine	1.08	0.68 ab	0.58 b	0.79 a	3.39 b	2.84 b	8.31 a
Valine	0.27	0.16 b	0.15 b	0.24 a	0.92 b	0.78 b	2.12 a
Methionine	0.15	0.09	0.10	0.11	0.38	0.38	0.73
Isoleucine	0.17	0.10 b	0.10 b	0.15 a	0.52 b	0.42 b	1.15 a
Leucine	0.32	0.21 b	0.23 b	0.30 a	1.03 b	0.95 b	2.05 a
Tyrosine	0.19	0.13	0.14	0.16	0.62 b	0.57 b	1.29 a
β-Alanine	0.08	0.05	0.05	0.05	0.42 ab	0.29 b	0.73 a
Phenylalanine	0.27	0.16 b	0.20 ab	0.23 a	0.75	0.79	1.49
Ornithine	0.05	0.04	0.06	0.05	0.13	0.23	0.32
Lysine	0.26	0.17 ab	0.13 b	0.21 a	0.88	0.63	1.86
Histidine	0.14	0.10	0.08	0.11	0.46	0.36	0.98
Anserine	0.92	0.61	0.55	0.59	3.73	3.00	5.97
Carnosine	29.51	23.18	21.13	22.40	130.90 b	109.71 b	223.21 a
Arginine	0.28	0.18	0.17	0.20	0.86	0.72	1.63
Total	37.00	28.75	26.23	28.68	159.95 b	133.35 b	279.80 a

[a] N = 15.
[b] N = 5. Where letters differ for a free amino acid in cooked meat, means differ significantly (P < 0.05) from each other (Duncan, 1955).
[c] N = 2–5. Where letters differ for a free amino acid in drippings, means differ significantly (LSD, P < 0.05) from each other.

Table 4—Mean free amino acid content (mg/g, dry, fat-free basis) of lamb roasts cooked by microwave and conventional methods

Free amino acid	Raw lamb[a]	Cooked lamb[b]			Drippings[c]		
		Microwave		Conventional	Microwave		Conventional
		220V	115V		220V	115V	
Taurine	7.74	5.08	5.47	5.95	39.46	43.40	44.05
Aspartic acid	0.61	0.33	0.30	0.45	1.08	1.28	1.40
Threonine	0.51	0.23	0.22	0.40	1.72 b	1.63 b	2.65 a
Serine	0.69	0.26 b	0.27 b	0.43 a	2.07 b	2.07 b	3.03 a
Glutamic acid	3.00	1.49 b	1.51 b	2.07 a	10.09	9.74	8.68
Proline	0.28	0.00	0.00	0.00	0.90 b	0.85 b	1.51 a
Glycine	0.71	0.40 b	0.36 b	0.53 a	3.12	2.89	3.23
Alanine	2.60	1.38 b	1.18 b	1.65 a	11.78	10.44	11.58
Valine	0.56	0.21 b	0.20 b	0.37 a	1.59 b	1.52 b	2.45 a
Methionine	0.40	0.15 b	0.15 b	0.27 a	0.88 b	0.91 b	1.46 a
Isoleucine	0.41	0.15 b	0.15 b	0.29 a	1.03 b	1.07 b	1.67 a
Leucine	0.93	0.34 b	0.36 b	0.71 a	2.16 b	2.35 b	3.81 a
Tyrosine	0.60	0.20 b	0.21 b	0.37 a	1.22 b	1.28 b	1.92 a
β-Alanine	0.00	0.00	0.00	0.00	0.08	0.07	0.13
Phenylalanine	0.58	0.23 b	0.24 b	0.43 a	1.15 b	1.24 b	1.89 a
Ornithine	0.16	0.09	0.08	0.12	0.82	0.77	0.97
Lysine	0.74	0.23 b	0.23 b	0.43 a	1.89 b	1.86 b	3.21 a
1-Methyl-histidine	0.15	0.10	0.07	0.13	0.88	0.63	0.98
Histidine	0.35	0.15 b	0.15 b	0.24 a	1.05 b	1.03 b	1.48 a
3-Methyl-histidine	0.03	0.03	0.03	0.03	0.17	0.20	0.33
Anserine	10.32	6.76	6.68	6.68	50.37	50.72	43.56
Carnosine	8.29	5.52	5.03	5.82	40.34	37.45	40.42
Arginine	0.82	0.33 b	0.30 b	0.51 a	2.42 b	2.25 b	3.34 a
Total	40.49	23.66 b	23.21 b	27.88 a	176.28	175.64	183.68

[a] N = 15.
[b] N = 5. Where letters differ for a free amino acid in cooked meat, means differ significantly (P < 0.05) from each other (Duncan, 1955).
[c] N = 2–5. Where letters differ for a free amino acid in drippings, means differ significantly (LSD, P < 0.05) from each other.

Table 5—Mean contents (μg/g) and percent retention of vitamins in roasts cooked by microwave and conventional methods

Species and vitamin	Cooked meat[a]			% Retention[a]		
	Microwave		Conventional	Microwave		Conventional
	220V	115V		220V	115 V	
Beef[b]						
Thiamine	0.89 a	0.74 b	0.92 a	61 a	49 b	69 a
Riboflavin	2.00	1.73	1.79	98 ab	83 b	99 a
Niacin	46.54	43.44	43.94	94 ab	86 b	104 a
Pork[c]						
Thiamine	14.78	14.48	17.51	73	67	72
Riboflavin	2.32	2.52	2.97	81	82	96
Niacin	47.31	59.06	46.01	87 ab	79 b	101 a
Lamb[d]						
Thiamine	1.96	2.10	1.93	52	49	52
Riboflavin	3.78	3.24	4.03	88	73	98
Niacin	44.56	36.67	43.14	71	64	86

[a] N = 5. Where letters differ within a vitamin for a species, means differ significantly (P < 0.05) from each other (Duncan, 1955).
[b] Raw beef: 1.06 μg/g thiamine, 1.45 μg/g riboflavin, 34.83 μg/g niacin. N = 15.
[c] Raw pork: 14.44 μg/g thiamine, 1.98 μg/g riboflavin, 37.13 μg/g niacin. N = 15.
[d] Raw lamb: 2.61 μg/g thiamine, 2.82 μg/g riboflavin, 37.21 μg/g niacin. N = 15.

Table 6–Mean mineral content (μg/g) and percent retention in roasts cooked by microwave and conventional methods

	Cooked meat[a]			% retention[a]			Drippings[a]		
	Microwave			Microwave			Microwave		
Species and mineral	220V	115 V	Conventional	220V	115V	Conventional	220V	115V	Conventional
Beef[b]									
Sodium	398	375	432	68	72	89	1461	1590	2090
Chloride	377	401	339	78	84	87	1426	1178	1616
Phosphorus	2162	2096	2086	75 b	79 b	98 a	4923 b	4738 b	6890 a
Iron	21	20	19	70 b	85 ab	98 a	25 b	24 b	34 a
Pork[c]									
Sodium	396	346	447	68	62	73	1157 b	934 b	2267 a
Chloride	422	387	420	74	68	70	1193 b	870 b	2418 a
Phosphorus	2368	2412	2589	74	70	76	5088 b	3964 b	9042 a
Iron	8	6	9	87	65	100	8 b	9 b	20 a
Lamb[d]									
Sodium	508 b	549 b	658 a	65	64	74	3281	2225	3105
Chloride	583	548	630	64	60	67	2280 b	1882 c	2655 a
Phosphorus	2286	2152	2279	77	78	92	6082 ab	5110 b	6888 a
Iron	21	20	24	73 b	79 b	110 a	30 b	20 b	61 a

[a] N = 5, except N = 4, for drippings of conventional method for lamb and pork. Where letters differ within a mineral for cooked meat or for drippings within a species, means differ significantly ($P < 0.05$) from each other (Duncan, 1955).
[b] Raw beef: 379 μg/g sodium, 337 μg/g chloride, 1889 μg/g phosphorus, 19 μg/g iron. N = 15.
[c] Raw pork: 381 μg/g sodium, 378 μg/g chloride, 2188 μg/g phosphorus, 7 μg/g iron. N = 15.
[d] Raw lamb: 581 μg/g sodium, 618 μg/g chloride, 1835 μg/g phosphorus, 17 μg/g iron. N = 15

uents were not affected significantly by the power level of the microwave range.

Data for moisture and protein contents of the three species of meat were similar to those presented by Watt and Merrill (1963) for comparable cuts. However, values lower than those of Watt and Merrill were found for the following constituents:

1. Fat content of cooked pork roasts.
2. Phosphorous content of cooked beef, pork and lamb.
3. Iron content of cooked beef and pork.
4. Niacin content of the three species of meat, particularly lamb.

Although these differences were apparent, increased use of microwave cookery for meat should cause no major concern for individuals with a diversified food intake. However, these data may be important to those with special dietary needs.

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Ms received 9/25/75; revised 12/13/75; accepted 12/16/75.

Contribution from the Missouri Agricultural Experiment Station. Journal Series No. 7389.

This investigation was supported by NIH research grant number 1R01 FD 00598-01 from the Dept. of Health, Education and Welfare, Public Health Service.

Appreciation is expressed to Sue Snider for assistance with the sensory panels and to Marc Jacob, Allen Foegeding, and Michael Glascock for assistance with the project.

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MECHANICALLY DESINEWED MEAT: ITS YIELD, COMPOSITION AND EFFECT ON PALATABILITY OF COOKED SALAMI

ABSTRACT

The effects of mechanical desinewing on yield, proximate analysis and connective tissue content of meat have been examined and its influence on the processing and palatability of cooked salami was evaluated. Mechanical desinewing removed approximately half the connective tissue and reduced the tendency for formation of gelatin pockets in cooked salami. Yields from the desinewer ranged from 71–87% and correspondingly higher cooking yields were obtained from desinewed meat. Protein and moisture increases were nonsignificant whereas the 2.39% change in fat content was, representing a 12.8% decrease. Tenderness and texture of cooked salami were improved by desinewing.

INTRODUCTION

MECHANICAL DEBONING of poultry for further processing has been widely practiced in emulsion-type products for years. Dhillon and Maurer (1975a, b) have recently shown that incorporation of some mechanically deboned chicken meat in summer sausages improved color and was acceptable to panelists. Inspection approval has stimulated interest in mechanically deboned red meat (Field et al., 1974a, b; Anderson and Gillett, 1974; Goldstrand, 1975), but the emulsionlike texture has limited its use primarily to emulsion products.

A specially designed head for the Beehive deboner permits the desinewing of cuts having heavy connective tissue. The desinewing head has larger perforations than the deboning head and the meat is extruded with a coarse texture allowing its use in salami and similar products. Yields, compositional changes and palatability properties of the mechanically desinewed meat are reported here.

EXPERIMENTAL

Experimental design

The experiment was designed according to Ostle (1963). A four by four factorial design was utilized to analyze the chemical composition of the starting ingredients, with four treatments (the control, texture control, desinewed meat and sinew), and four cuts (the shank, plate chuck, and pork shoulder). A three by three factorial design allowed evaluation of the composition and panel data on salami made from the three beef cuts (the shank, plate, and chuck, each combined with pork) and three treatment groups (the control, texture control, and desinewed meat).

Table 1–Spice, cure and other ingredients in salami formation

Ingredient	Amount kg/100 kg Meat
Griffith's Regal salami seasoning	1.00
Garlic	0.27
Black pepper	0.16
Salt	2.01
Sodium nitrite	150 ppm
Griffith's FOS-5 (Polyphosphates)	0.75

Sample preparation

Sixty-four pork shoulders (U.S. No. 2), 10 chucks, 48 shanks, and 48 plates from utility grade beef cattle were used in the study. Paired cuts from corresponding sides were identified and each hand boned.

Meat from one side was ground once through a 1.27 cm plate, once through a 0.95 cm plate, divided in half and one-half was used as the control. The control would be similar in coarseness to that used in commercial practice. The remaining half, which was subsequently ground through a 0.31 cm plate, was the texture control. Its particle size was similar to the mechanically deboned meat whereas the control was coarser. Meat from the other side of the carcass was ground through a 2-in. plate and desinewed with a Beehive AUX 1272 deboner with a desinewing head containing 0.19 cm perforations. The desinewed meat was immediately chilled with CO_2.

Samples were taken from each of the controls and the desinewed fraction, reground twice through a 0.31 cm plate, frozen and stored for subsequent analysis. The remainder of the meat from each fraction was frozen at −28.8°C and held for product formulation.

Proximate analysis

Fat, protein, moisture and ash contents were analyzed by AOAC methods (1965). Proximate analyses were made in triplicate on the starting ingredients and in duplicate on salami products.

Connective tissue

Samples were hydrolyzed as described by Woessner (1961). Hydroxyproline contents were determined by the method of Bergman and Loxley (1963) using a Spectronic 20, Bausch and Lomb spectrophotometer. Connective tissue contents were calculated from hydroxyproline values as described by Wyler (1972).

Salami preparation

Salami prepared for taste panel evaluation was formulated to contain 60% beef and 40% pork with a proximate analysis of about 15% protein, 20% fat, 60% moisture and 5% ash for the final products. The salami formula, cure, spice and other ingredients are shown in Table 1. Polyphosphate (0.75%) and a lower than normal level of added water were necessary to prevent gel pockets in salami made from the nondesinewed controls so panel comparisons could be made. Therefore, products made from desinewed and nondesinewed meat which were presented to the panels contained the polyphosphate. Meat for the formulation of products was from the control, texture control or mechanically desinewed fractions of beef cuts and from similar fractions of the pork shoulder. Fat levels were adjusted by adding some cutting fat from choice beef carcasses. Moisture levels were regulated by adding water. All ingredients were mixed for 3 min in a small mixer, stuffed into presoaked 8U (14.1 cm flat width) fibrous cellulose casings, and held overnight at 4–6°C. The salami were then cooked in a light smoke for 2 hr at 49°C, 2 hr at 54°C and 1 hr at 65.5°C. The temperature was then elevated to and held at 82°C until the products reached 71°C. After removal from the smokehouse a cold shower was applied until the internal temperature declined to 49°C. The products were allowed to bloom and dry at room temperature for 45 min prior to chilling.

Taste panel

A trained taste panel of 21 members evaluated the salami comparing the control, texture control and desinewed fractions. Tenderness, texture, appearance, and overall acceptability were scored on a 9-point hedonic scale ranging from extremely coarse (1) to extremely fine (9) for texture; from extremely tough (1) to extremely tender (9) for tenderness; from extremely dry (1) to extremely juicy (9) in juiciness;

Table 2–Mean comparisons of chemical components of hand boned wholesale cuts[a]

Proximate composition	Wholesale cut means: Beef Shank	Beef Chuck	Beef Plate	Pork shoulder	MSE	LSD (P < 0.01)
% Protein	19.79a	17.78b	17.35b	13.67c	0.3653	0.39
% Fat	14.05a	15.01b	24.64c	32.02d	1.4040	0.77
% H_2O	65.36a	65.86a	57.09b	53.87c	2.8192	1.09
% Ash	0.886a	0.895a	0.776b	0.736c	0.0064	0.05
% Hydroxyproline	0.573a	0.364b	0.368b	0.247c	0.0014	0.02
% Connective tissue	4.58a	2.92b	2.95b	1.98c	0.0903	0.19
% Connective tissue on fat free basis	33.87a	19.45b	11.81c	5.95d	14.1402	2.44

[a]Means on the same line followed by different letters are significantly different (P < 0.01).

and from dislike extremely (1) to like extremely (9) for overall acceptability and appearance.

Statistical analysis

The least significant difference method (LSD) was used as the method of multiple mean comparison following a significant F ratio in the standard analysis of variance (Ostle, 1963).

RESULTS & DISCUSSION

Desinewing

Yields from the desinewer ranged from 70.9% for beef plates to 87.2% for pork shoulders. Beef shanks yielded 80.6% while chucks were 86.1%. Temperature increases during grinding and desinewing were approximately 3°C (1–4°C) for the beef chucks, plates, and pork shoulders and 5°C for the beef shanks. The greater increase in temperature and lower yields for the shanks were probably due to the heavier connective tissue in that cut. The temperature increase during desinewing was less than for mechanical deboning (–1 to 11°C) of mutton carcasses (Field et al., 1974b) and should present little problem if carbon dioxide chillers were used to lower the temperature immediately.

Proximate analyses of cuts

Table 2 shows the mean protein, fat, moisture, ash, hydroxyproline and connective tissue contents of meat from the four wholesale cuts. The shank was significantly higher in protein than all other cuts while the pork shoulder was lowest (P < 0.01). There was no significant difference between the chuck and plate in percent protein. Fat varied, inversely with protein on the four cuts and each varied significantly (P < 0.01) from all other cuts. Pork shoulders were highest in fat (32.02%) while beef shanks were lowest (14.05%).

Moisture levels were similar for shank and chuck (65.36 vs 65.86%) and were significantly higher (P < 0.01) than the plate or pork shoulder. The pork shoulder was significantly lower (P < 0.01) than all other cuts (53.87%).

Ash levels were comparable in the shank and chuck but lower in the plate and shoulder, probably due to the higher fat content of the latter two cuts. Hydroxyproline and connective tissue were highest in the shank, intermeidate in the chuck and plate, and lowest in the pork shoulder (P < 0.01). On a fat-free basis, all four cuts varied significantly in percent connective tissue (P < 0.01). The shank was highest (33.87%) followed by the chuck (19.45%), plate (11.81%) and pork shoulder (5.95%).

Table 3–Factorial analysis of the effects of mechanical desinewing upon various meat components from beef shank, plate and chuck and pork shoulders[a]

Item	Treatment means: Control and texture control[b]	Desinewed meat	Sinew fraction	MSE	LSD (P < 0.01)
% Protein	16.99a	17.22ab	17.40b	0.3653	0.39
% Fat	18.66a	16.27b	32.14c	1.4040	0.77
% H_2O	63.28a	65.45b	50.16c	2.8193	1.09
% Ash	0.87a	0.90a	0.66b	0.0064	0.05
% Hydroxyproline	0.36a	0.20b	0.63c	0.0014	0.02
% Connective tissue	2.91a	1.56b	5.05c	0.0903	0.19
% Connective tissue in protein	16.75a	9.01b	28.45c	2.8925	1.10
% Connective tissue on fat free basis	20.68a	12.53b	17.20c	14.1402	2.44
Ratio H_2O to connective tissue	23.95a	44.29b	10.68c	6.8391	1.70

[a]Means on the same line followed by different letters are significantly different (P < 0.01).

[b]Texture control samples were reground through a 0.31-cm plate to make particle sizes comparable to mechanically desinewed meat.

Effect of desinewing on composition

Mechanical desinewing did not significantly alter (P < 0.01) the percent protein or percent ash in the meat (Table 3), however, the percent decrease in fat was 12.8% and the percent decrease in hydroxyproline and connective tissue was 46% (wet basis). Calculated on a fat-free basis, desinewing reduced the percent hydroxyproline and connective tissue by over 39%. This left the desinewed fraction with 9.01% of its protein as connective tissue, and represented less than one-third the connective tissue content of the sinew fraction (protein basis). Moisture levels in the desinewed fractions were slightly higher, likely due to the reduced fat levels.

Highly significant cut by treatment interactions occurred in both hydroxyproline and connective tissue indicating that inherent differences in cuts affect the removal of connective tissue upon mechanical desinewing. Table 3 illustrates the mean percent hydroxyproline in the four cuts before and after desinewing and also includes those of the sinew fractions.

Figure 1 illustrates the practical significance of the removal of the connective tissue from the meat. The nondesinewed samples broke down and formed jelly pockets at the bottom of the casings (right end in figure). In contrast the desinewed meat, processed under identical conditions, did not separate and jelly pockets were not visible. Also of some significance to processing is the H_2O/connective tissue ratio (Table 3). The desinewer changed the ratio from 23.95 to 44.39 which is undoubtedly associated with the reduced shrinkage and absence of jelly pockets in the products containing desinewed meat. In processing the control samples for texture and tenderness evaluation, it was necessary to add 0.75% polyphosphate and reduce the added water to prevent the formation of jelly pockets. Shrinkage was largest on the nondesinewed salami (Fig. 2) as evidenced by the wrinkled appearance of the casing, despite the phosphate and reduced water.

Composition and cooking yield of salami products

Compositions and cooking yields of the final salami prod-

ucts are shown in Table 4. There were no significant differences in any of the proximate analyses ($P < 0.01$) between treatments or cuts. Cooking yields are from a single experiment but suggest that the size of the grind had an effect upon yield. Mechanically desinewed meat gave the highest cooking yields.

Panel evaluation of salami from various cuts

Table 5 contains mean panel scores for texture, tenderness, juiciness, appearance and overall acceptability of salami made from beef shanks, plates, or chucks combined with meat from pork shoulders. Significant differences in palatability between salami made from these cuts did not occur ($P < 0.01$). The single exception was in overall acceptability where beef chuck proved inferior to shank and plate. There was no apparent reason for the lower mean panel values on palatability characteristics of meat containing chuck and the differences were not large.

Panel evaluation of salami or desinewed vs nondesinewed meat

Mean panel scores given salami made from cuts involving different treatments are found in Table 6. Significant differences between the coarseness of grind and palatability characteristics did not occur as shown by comparing the control and texture control treatments ($P < 0.01$). A grind similar in coarseness to mechanically desinewed meat did not alter the palatability characteristics of salami. Highly significant differences ($P < 0.01$) did occur in texture and tenderness between salami made from the controls and from the desinewed meat. This confirms the fact that mechanical desinewing of meat, which removed approximately half of the connective tissue improves texture and tenderness.

No significant differences in juiciness ($P < 0.01$) occurred when control samples were compared to desinewed samples, even though mean values were higher on the desinewed salami products. Associated higher moisture contents as determined by oven drying are in agreement with the juiciness values obtained by taste panel evaluations and showed that the desinewed meat was only slightly higher in moisture and/or juiciness than the controls.

Appearance and overall acceptability of salami made from desinewed meat was preferred over the coarse ground control, but not over the more finely ground texture control ($P < 0.01$). The coarse appearance of the fat in the salami was

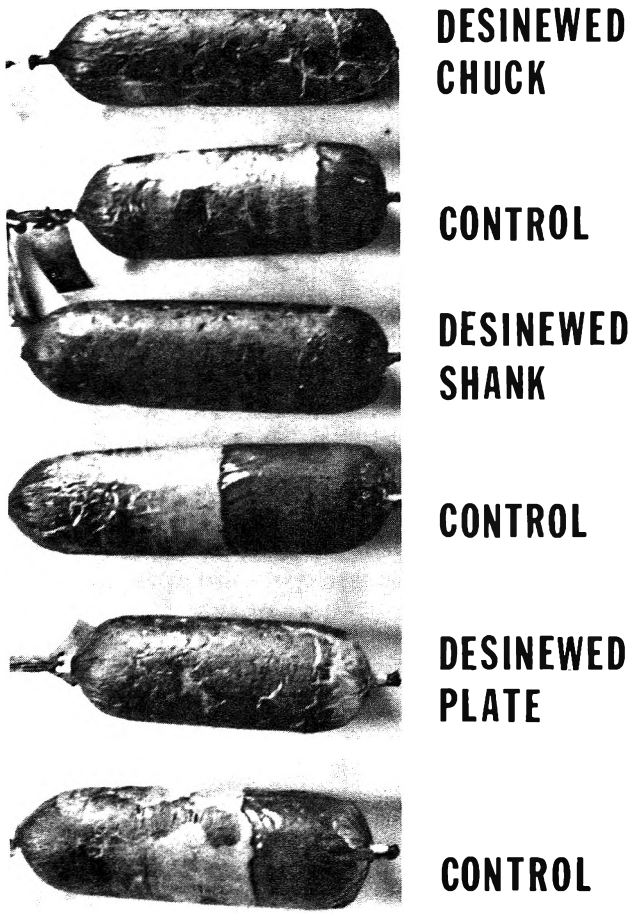

Fig. 1—Effect of mechanical desinewing upon the formation of gelatin pockets is shown above. Salami was processed from frozen desinewed beef chuck, shank, and plate where 60% of the meat was from one of the beef cuts and 40% from pork shoulders with identical treatment. 20% added water was incorporated and the spice formulation was identical to Table 1 except polyphosphates were not included.

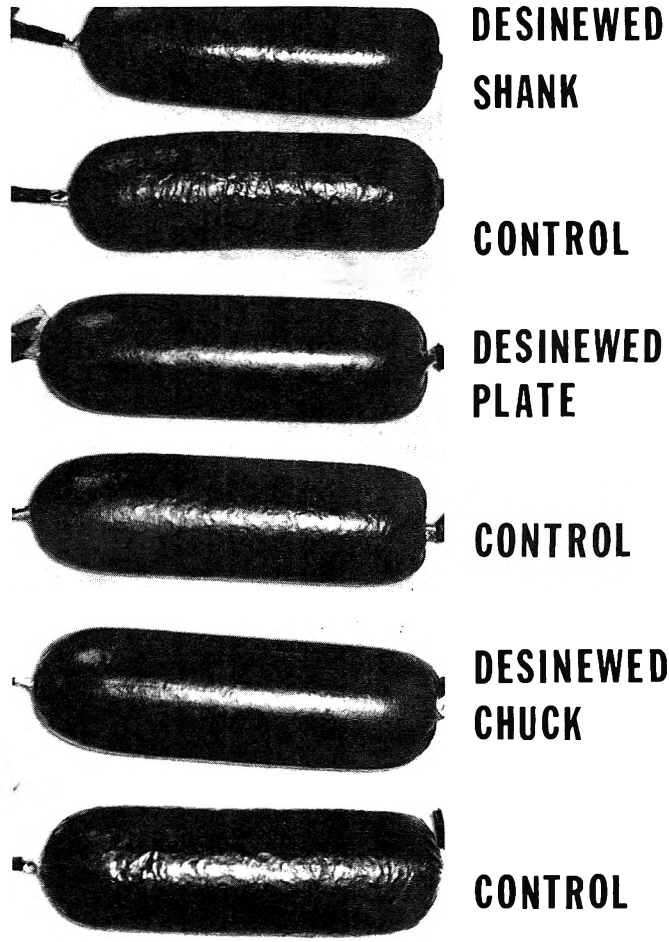

Fig. 2—Polyphosphates and reduced water levels were used to prevent formation of jelly pockets but, shrinkage is noted by presence of wrinkled casings on nondesinewed salami.

Table 4–Mean values obtained for proximate analysis[a] and cooking yield on salami products[b]

Cuts and treatments	Moisture (%)	Fat (%)	Protein (%)	Cooking yield (%)
Shank control	60.20	19.23	16.59	86.04
Shank texture control	60.55	18.14	17.00	91.01
Shank desinewed	57.91	20.32	15.12	91.35
Plate control	57.02	20.78	15.00	86.68
Plate texture control	59.57	19.18	14.92	93.05
Plate desinewed	60.71	18.39	14.70	93.57
Chuck control	58.11	21.24	14.35	86.83
Chuck texture control	57.10	21.00	14.90	89.80
Chuck desinewed	60.99	18.50	14.51	93.10

[a] Analysis of variance revealed no significant difference in proximate analysis ($P < 0.01$).
[b] Single values without statistical analysis

Table 5–Mean taste panel scores on palatability characteristics of salami as affected by source of cut[a]

Palatability characteristic	Cut			LSD ($P < 0.01$)
	Shank	Plate	Chuck	
Texture	5.03a	5.44a	4.59a	0.99
Tenderness	5.41a	5.90a	5.48a	0.65
Juiciness	5.44a	5.63a	5.13a	0.48
Appearance	4.51a	5.02a	4.03a	1.30
Overall	5.39a	5.71a	4.57b	1.01

[a] Means on the same line followed by different letters are significantly different ($P < 0.01$).

Table 6–Mean taste panel scores on palatability characteristics of salami as affected by coarseness of grind and mechanical desinewing[a]

Palatability characteristic	Treatment			LSD ($P < 0.01$)
	Control	Texture control[b]	Desinewed meat	
Texture	4.11b	4.98b	5.98a	0.99
Tenderness	5.02b	5.56b	6.21a	0.65
Juiciness	5.25a	5.26a	5.69a	0.48
Appearance	3.45b	4.44ab	5.66a	1.30
Overall	4.52b	5.20ab	5.96a	1.01

[a] Means on the same line followed by different letters are significantly different ($P < 0.01$).
[b] Texture control meat was reground through a 0.31 cm plate to make particle size comparable to mechanically desinewed meat.

objectionable to panelists and undoubtedly accounted for its lower mean scores in appearance and overall acceptability for the control salami.

CONCLUSIONS

MECHANICAL DESINEWING removed approximately half of the connective tissue from beef and pork trimmings and under certain conditions should improve meat for use in processed products. The tendency for jelly pockets to form in salami was reduced, and its texture and tenderness were improved by the desinewing operation. Changes in proximate analyses occurred but appeared minor in nature except for fat which had a percent decrease of 12.8%. Yields were relatively high from the desinewer and correspondingly higher cooking yields would be expected from desinewed meats. The reduction in connective tissue content caused by mechanical desinewing meat may affect the protein efficiency ratio. This possibility is currently under investigation. It would be possible to render edible fat from the sinew fraction following mechanical desinewing. It may also be possible to obtain edible collagen and other proteins from this fraction.

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Ms received 8/22/75; revised 12/26/75; accepted 12/29/75.

Appreciation is expressed for the laboratory assistance of Russell Reeder and Ferris Garrett.

Use of the mechanical desinewer was contributed by Beehive Machinery, Salt Lake City, Utah.

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ELECTRODIALYSIS OF RAW WHEY AND WHEY FRACTIONATED BY REVERSE OSMOSIS AND ULTRAFILTRATION

ABSTRACT

Electrodialysis is a unit operation which removes ionic species from a solution through the use of an imposed voltage and ion-selective membranes. The temperature, flow rate, and composition of the feed, and the applied voltage all influence the rate of ionic transport in electrodialysis systems. The effects of the aforementioned process parameters on the performance of an electrodialysis system were investigated using cottage cheese whey as the process feed. Increasing the flow rate proved to be of little value in decreasing the membrane stack resistance for a given run, but it significantly retarded the rate at which the stack resistance increased in repeated runs. The effect of increasing the total solids concentration of the whey without altering the solute composition was to increase the potential rate of demineralization through an increase in conductivity. Prefractionation of the feed by ultrafiltration enhanced the value of the whey solids by increasing the protein concentration and decreasing the ash concentration. However, the electrodialytic process was less efficient in removing the remaining ash in this case than it was in demineralizing other feeds.

INTRODUCTION

ANNUALLY, 26–27 billion pounds of cheese whey other than cottage cheese whey are produced in the United States (Lough, 1974). In addition, the production figures indicate that 9–10 billion pounds of cottage cheese whey were produced in 1973. Thus, the total whey production is in excess of 36 billion pounds containing more than 250 million pounds of protein. Approximately 50% of this whey is discarded as waste (Groves, 1973). This practice not only negates the potential use of whey as a nutritional source, but also presents a pollution control or waste treatment problem since the BOD (biological oxygen demand) content of the whey is high (Chan and Selldorff, 1969). One common type of whey is cottage cheese whey, and the problem of finding uses for this material provided the impetus for the study discussed herein.

The reasons why the uses of raw whey are rather limited become evident from an examination of the solids contained therein (Table 1). (Hereafter, "whey" refers to cottage cheese whey.) The three major solutes in whey are lactose, ash, and protein. The protein content is an asset because protein is one of the most expensive and most deficient nutrients in the world's food supply. However, the ash and lactose concentrations must be regarded as liabilities. Lactose has low solubility, low sweetening power, and undesirable laxative effects if used in large amounts in the mammalian diet. The ash content places palatability limits on the amounts of whey that can be tolerated in the human diet.

The problems associated with whey utilization have drawn considerable research interest in the past several years. A possible solution that has been suggested for the lactose utilization problem is to use the lactose as a medium for growing yeast thereby producing protein (Marth, 1970). However, this paper is concerned with the problem of ash removal, specifically the demineralization of whey by electrodialysis. Both raw whey and wheys that had been preconcentrated or prefractionated by reverse osmosis or ultrafiltration were used as feeds to the electrodialysis unit.

The salient features of the reverse osmosis, ultrafiltration, and electrodialysis processes are shown in Figure 1 (a, b, c, d).

Reverse osmosis and ultrafiltration are pressure-driven membrane processes. They remove selective species from solution with the aid of a semipermeable membrane.

Figure 2 shows the basic effect of the pressure driven membrane process on the composition of the whey. For all practical purposes, reverse osmosis removes only water and the effect is to increase the total solids concentration of the whey without affecting the relative amounts of the various solutes. Ultrafiltration membranes are impermeable to protein but are permeable to low molecular weight solutes and to water. Both lactose and ash are removed with the water in this process. Thus as ultrafiltration proceeds, the relative amounts of the various molecular species present in the retentate will vary. The concentration of the protein and other high molecular weight species will be enhanced relative to low molecular weight species like lactose and the ash constituents. The compositions of the retentate at various volume reductions are listed in Table 2.

Electrodialysis is a unit operation by which ionic species are removed from a solution with the aid of anion and cation selective membranes and an imposed voltage. A good discussion of the process is given by Mintz and Shaffer (1966). An alternating sequence of anion selective and cation selective membranes across which a voltage is imposed can be used to split a feed stream into two fractions, one enriched and the other depleted in ionic species. It is also possible to bring about a reduction in ionic content by using a sequence of cation selective and neutral membranes. This process (termed "transport depletion") operates on basically the same principles and is also discussed by Mintz and Shaffer (1966) and Kollsman (1959).

There are two major constraints on the operation of most electrodialysis systems: the "limiting voltage" and the "limiting current."

The limiting voltage is generally given by an empirical correlation. This allowable voltage decreases with increasing conductivity in the feed stream so the net effect is that, regardless of the feed conductivity, the current which passes through the stack at the limiting voltage is quite constant. This situation is a result of the problem which gives rise to the empirical relationship, namely the necessity for keeping the current below the value where excessive heating of the system results in the melting of parts of the plastic flow channels.

The limiting current is a consequence of a phenomena known as "concentration polarization." This phenomena is discussed extensively by Mintz and Shaffer (1966). In essence, the limiting current is a measure of how well the solution can transport charge and is approximately proportional to the conductivity of the solution to be demineralized.

Both the limiting voltage and the limiting current govern the maximum amount of current that may be passed through the system. This fact is important because it is generally these constraints and not economic factors that limit the current that can be used in electrodialytic processes. The cost of electrical energy in most electrodialysis systems is dwarfed by the cost of membrane replacement and membrane-area related costs such as labor and buildings.

Since both constraints affect the allowable current, only one is active at any given time. As mentioned above, the current allowed by the limiting voltage is relatively constant for a variety of feed conditions. The limiting current increases approximately linearly with conductivity. Thus, at high conductivities, where the limiting current exceeds the current allowed by the limiting voltage, the system will be "voltage-limited," and at lower conductivities the system will be "current-limited."

EXPERIMENTAL

THE ELECTRODIALYTIC DEMINERALIZATION was carried out in a unit supplied by Ionics, Inc. of Watertown, Mass. There were three separate streams as indicated in Figure 3. The mode of operation was batch recirculation and the 9 in. × 10 in. membrane stack contained 18 cell pairs. Whey was circulated in both the concentrate and diluate streams, so at the end of a run the concentrate tank contained high-ash whey to be discarded and the diluate tank contained demineralized whey to be retained as product. In a commercial installation, a salt solution with a conductivity comparable to whey would be used in the concentrate stream. Whey was used in our experiments strictly for convenience.

The temperature, voltage, and flow rate were maintained at constant values throughout the course of a given run. Samples were taken from the product tank before the start of each run, from the effluent from the stack at given intervals, and then again from the product tank at the end of the run. Analyses for ash content were by combustion at 550°C. In order for the plots of the fraction demineralized versus time to be

Table 1—Composition of dried cottage cheese whey[a]

	(%)
Moisture	2.5
Solids	97.5
Lactose	63.6
Ash	11.6
Protein	13.3
Fat	0.6
Acidity as lactic acid	4.5

[a] Weisberg and Goldsmith, 1969

Table 2—Composition of whey after preconcentration by ultrafiltration

Volume Reduction	0	50%	67%	80%	90%
Total solids (%)	5.92	6.72	7.66	11.03	15.78
Wt % ash (Wet basis)	0.65	0.64	0.69	0.78	0.85
Wt % protein (Wet basis)[a]	0.74	1.28	1.80	3.34	6.59
Est wt % lactose (Wet basis)	3.8	4.0	4.2	5.5	6.3
Wt % ash (Dry basis)	11.2	9.6	9.0	7.1	5.4
Wt % protein (Dry basis)	12.5	19.1	23.5	30.2	41.8
Est wt % lactose (Dry basis)	65	60	55	50	40

[a] Total nitrogen X 6.38

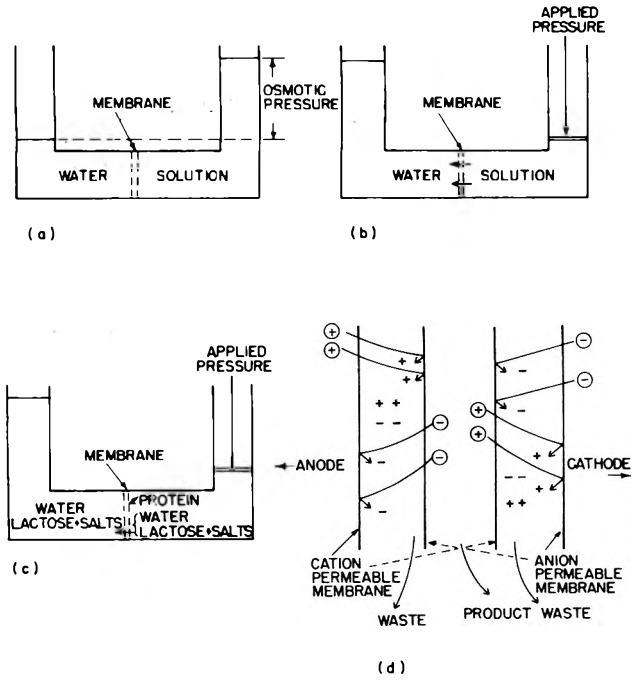

Fig. 1—(a) Equilibrium situation, no osmotic flow; (b) Reverse osmosis: Membrane rejects all solutes; (c) Ultrafiltration of whey: Water and microsolutes can pass through the membrane, macrosolutes are rejected; (d) Electrodialysis.

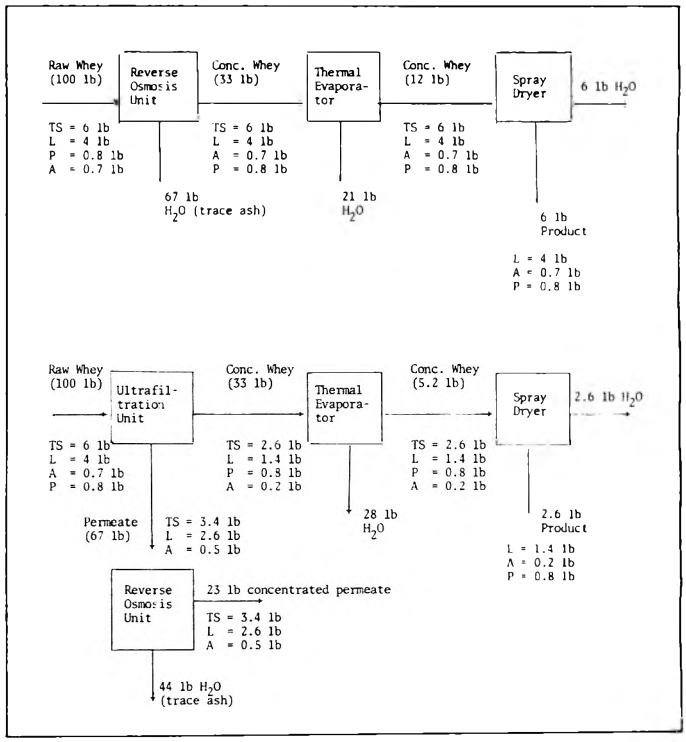

Fig. 2—Processing schemes utilizing reverse osmosis, ultrafiltration and thermal evaporation. (TS = Total solids, L = Lactose, P = Protein, A = Ash.)

meaningful, the time axis was adjusted for the effluent samples so that all measured ash concentrations represented tank concentrations.

The membranes used in the electrodialysis experiments were Ionics 110BZL-219 and 111EZL-219 anion selective membranes and Ionics 61CZL-813 cation selective membranes obtained from Ionics, Inc., Watertown, Mass. The reverse osmosis and ultrafiltration membrane systems were obtained from the Fluid Sciences Division of Universal Oil Products, San Diego, Calif.

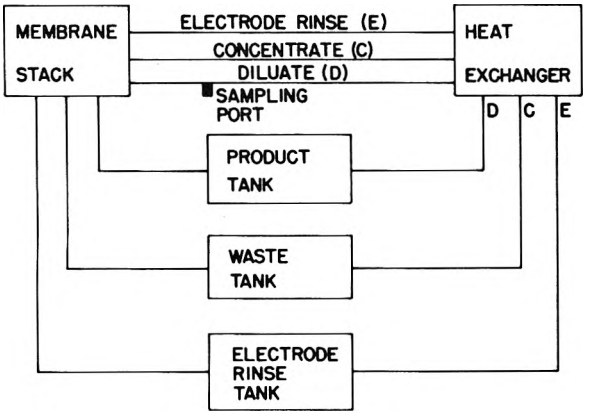

Fig. 3—Schematic diagram of experimental system.

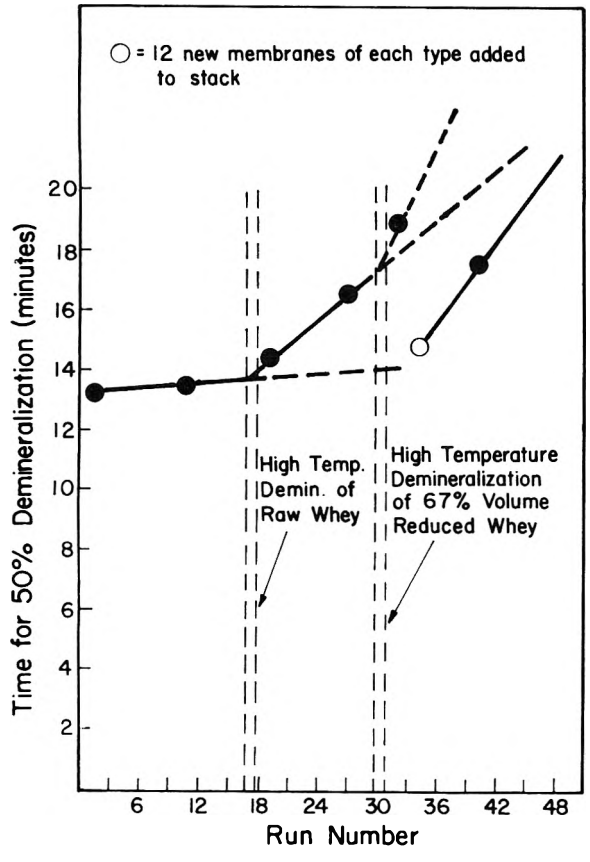

Fig. 4—Effect of time on stream on the time required for the 50% demineralization of raw cottage cheese whey. (Temperature = 26–29° C; Voltage = 76–78 volts; Flow rate = 20 ml/sec.)

RESULTS & DISCUSSION

THROUGHOUT the experiments, the time required for a given degree of demineralization for a particular set of conditions continually increased. This was due to increasing membrane resistance as evidenced by current readings. Figure 4 shows how the time required for the 50% demineralization of raw cottage cheese whey varied with the number of runs for which the membranes had been used. This type of run was carried out periodically in order to characterize the condition of the membranes. The other experiments conducted involved many comparisons, and for these comparisons to be accurate the condition of the membranes had to be known.

The information contained in Figure 4 described the conditions of the membranes as completely as was necessary for all important calculations. The most important calculations in all analyses were the rate of demineralization and the energy consumed. Rates for different runs could be accurately compared by noting the condition of the membranes for each run as shown in Figure 4 and then adjusting the rates accordingly. It was found that the energy consumed in demineralizing raw whey was not affected by the decrease in the demineralization rate (increase in membrane resistance) with respect to time. It was assumed that this was true in all systems where membrane age was the only variable. Therefore, energies of demineralization were used without adjustment regardless of membrane condition.

Evaluation of operating constraints for experimental system

As the discussion in the introduction indicates, the two major operational constraints in electrodialysis systems are the limiting voltage and the limiting current. Therefore, it was important that these constraints be determined for the experimental system.

Once the conductivity of the fluid to be demineralized was determined, the limiting voltage was calculated from an empirical relationship supplied by the manufacturer. The limiting voltage is given by Equations 1 and 2 (Ionics, 1967).

$$V_{max\ 64} = (1.4 + 0.02R)n \quad (1)$$

$$\frac{V_{max}}{V_{max\ 64}} = \frac{1}{1 + 0.01(T\text{-}64)} \quad (2)$$

where V_{max} = the limiting voltage across n cell pairs at an arbitrary temperature; $V_{max\ 64}$ = the limiting voltage across n cell pairs at 64°F; T = temperature, degrees Fahrenheit; R = Resistivity of most conductive stream, Ohm-cm; and n = number of cell pairs. As expected, regardless of feed conditions, when the appropriate limiting voltage was applied, the current varied over approximately the same range (initial value of 4–4.5 amps; final value of 2.2–2.7 amps).

The polarization point for raw whey was found to occur at a current between 9 and 10 amperes. [See Sata et al. (1969) for a discussion of how to determine the polarization point.] Only one polarization run was necessary because of the range of conductivities that were encountered in the experiments. The lowest conductivity encountered was 5500 microhms/cm (18°C) for whey that had undergone a 90% volume reduction by ultrafiltration and the highest was 10,600 microhms/cm for whey that had been concentrated to 18.24% total solids by reverse osmosis. To a first approximation, the limiting current is porportional to the conductivity (Ionics, Inc., 1967). Since the conductivity of raw whey at 18°C was approximately 6,300 microhms/cm, these conductivity readings implied that the limiting current either increased or decreased only slightly over the range of feed conditions encountered. As previously mentioned, the current at the limiting voltage varied over a

relatively narrow range, and this range was well below the limiting current for raw whey. As a result, it was assumed that the limiting current would not be exceeded in any of our experiments without exceeding the limiting voltage, i.e., that we would be voltage limited.

Effect of voltage

The curves showing the fraction demineralized as a function of time for raw whey were plotted for the various voltages tested (see Fig. 5). The limiting voltage for raw whey was 76–78 volts and at 125 volts the initial value of the current approached the limiting current. The heat exchange capacity was such that there were no problems of heat build up in the stack. The energy consumption for 50% demineralization at each voltage was calculated by integrating the current over time and multiplying by the voltage. The results are tabulated in Table 3.

It is important to note that the ratio of the energy consumed to the voltage varies by only three to four percent. This observation implies that, for a given degree of demineralization, the integral of current over time (related to current efficiency) is a constant over a considerable range of voltage.

Effect of flow rate

The costs that are proportional to membrane area are by far the most important costs encountered in electrodialysis systems. Therefore, conditions which tend to reduce the amount of membrane area necessary for a given degree and rate of demineralization are highly desirable. The performance of the electrodialysis system at high flow velocities was studied because of two possible effects that would decrease the amount of membrane area necessary to achieve a given degree of demineralization. First, a higher flow rate generally produces a higher degree of turbulence and thinner hydrodynamic boundary layers. Since a significant part of the stack resistance can be traced to the ion-depleted boundary layers (Mintz and Shaffer, 1966), operation at high fluid velocities could result in a substantial decrease in stack resistance and an increase in demineralization rate (decrease in required membrane area) for a given voltage. Secondly, high flow rates inhibit membrane fouling. Since the boundary layer is less resistive at high flow rates, transport of H^+ and OH^- ions and concomitant pH changes will be less marked, and protein precipitation will be less likely. Also the high flow rates may have a scrubbing effect on the membrane surface, sweeping away proteins and colloidal particles that might otherwise foul the membranes. The desirable effect of reduced membrane fouling would be to decrease the frequency of system shutdown for cleaning.

In our system, the flow rate had little effect on the boundary layer resistance as evidenced by the fact that a 250% increase in flow rate at a given voltage had virtually no effect on the rate of demineralization (see Fig. 6). This observation may be attributed to the fact that the flow conditions at the lower flow rate were already well within the turbulent regime due to the turbulence promoters in the flow path. This result was not totally unexpected since D'Souza (1972) and D'Souza et al. (1973) found that a 30% change in flow rate had little effect on the rate of demineralization for the same system. There was, however, good evidence of a scrubbing effect at high flow rates. At the higher flow rate the rate of membrane fouling was significantly reduced.

The major drawback to the use of high flow rates is the concomitant increase in pumping costs. The 250% increase in flow rate was made possible only through a 400–500% increase in the stack pressure drop. However, the energy consumed in pumping was only a small fraction ($\sim$1%) of the total energy consumed by the process and an even smaller fraction of the total production cost. In light of the advantages to be gained in the efficient utilization of membrane area, the use of high flow rates is recommended for the electrodialysis of feeds containing materials which give rise to extensive fouling.

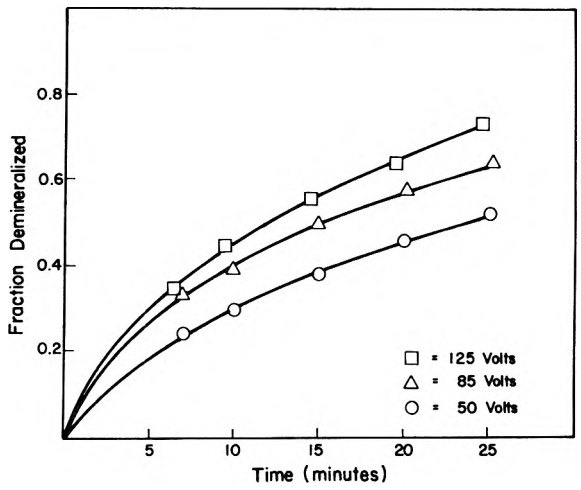

Fig. 5—Effect of voltage on the rate of demineralization of raw cottage cheese whey. (Temperature = 26–29° C; Flow rate = 20 ml/sec.)

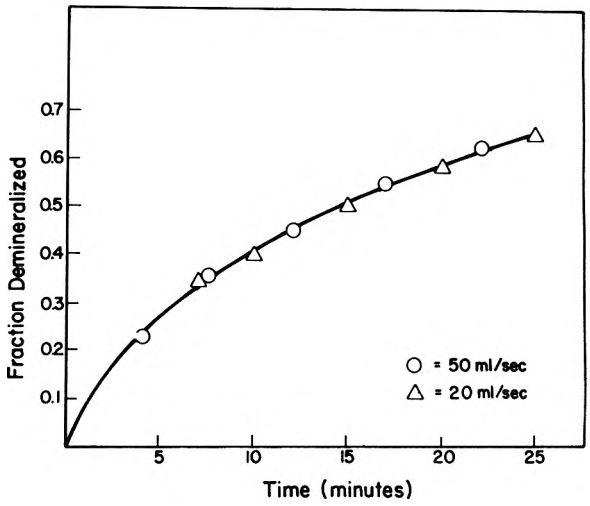

Fig. 6—Effect of flow rate on the rate of demineralization of raw cottage cheese whey. (Temperature = 26–29° C; Voltage = 85 volts.)

Table 3—Effect of voltage on the energy consumed in 50% demineralization of raw cottage cheese whey[a]

Voltage	Run no.	Energy (Joules)	Average (Joules)	Average Voltage (Coul.)
50–52	1	2.57×10^5	2.67×10^5	5240
	2	2.67		
	3	2.77		
85–87	1	4.67×10^5	4.66×10^5	5420
	2	4.53		
	3	4.80		
125–127	1	6.55×10^5	6.60×10^5	5420
	2	6.64		

[a] System volume = 7.16 liters (product stream); initial ash conc approx 0.65% by weight (wet basis)

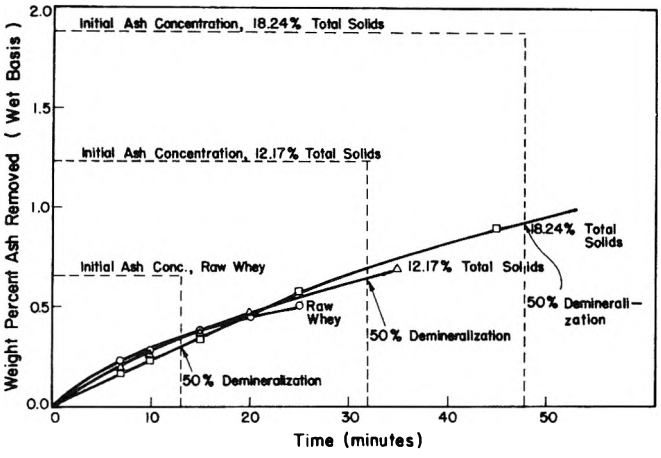

Fig. 7—Demineralization curves for whey preconcentrated by reverse osmosis.

While the electrodialysis of raw whey has been described in patents (Francis, 1969, 1971; Francis and Treleven, 1970, 1973; Scheder, 1970, 1971a, b, c, d, 1972; Wang, 1975), comparison of the above results with the patent descriptions is not meaningful because of the fundamental character of the patent literature.

Effects of preconcentration of feed

The effects of feed concentration were analyzed for two different concentration schemes. The discussion will be divided into two corresponding sections, one pertaining to preconcentration by reverse osmosis and the other to preconcentration by ultrafiltration.

Since reverse osmosis has little effect on the composition of the whey solids, the product value is independent of the feed concentration and the cost of production per unit weight of ultimate dry product as a function of feed concentration is a meaningful objective function. However, feed preconcentrated by ultrafiltration varies in protein concentration and, thus, in value. The cost of production per unit weight of ultimate dry product is meaningful only in the light of the product value.

Preconcentration by reverse osmosis

The experiments conducted to determine the effects of preconcentration by reverse osmosis on the electrodialysis process included the demineralization of raw whey and whey that had been concentrated to 12.17% total solids and 18.24% total solids. The demineralization curves are shown in Figure 7.

The first thing to notice in Figure 7 is the shape of the curves. In each case, the rate of demineralization declines as time progresses. This was to be expected because the voltage was always held constant throughout the course of a run but the stack resistance increased due to the demineralization of the product stream.

The second thing to notice is that the curves for all feed concentrations are initially very close together. This was to be expected because the system was voltage-limited, resulting in a fairly constant initial current over the range of feed concentrations.

A final thing to notice is that the rate at which the slope of the curve changes decreases as the feed concentration increases. This was also expected because a given amount of ash removal represented a smaller degree of demineralization as the feed concentration increased. Thus it took longer for the product stream conductivity to change appreciably from its initial value.

The essence of the three observations mentioned above is that all of the curves had nearly the same shape in relation to their respective asymptotes (initial ash concentrations). Therefore, the average rate of ash removal for a given percent demineralization was fairly constant over the range of feed concentrations studied. The initial currents were roughly the same for all feed concentrations as were the currents after 50% demineralization, a fact which points to the same conclusion.

Since the rate of ash removal was fairly constant and the amount of ash removed per pound of ultimate dried product was uniform over the range of feed concentrations, the rate of production of demineralized whey on a dry basis was also quite constant, ranging from 68–76 lb per day for the test apparatus used in the present study.

There are two important conclusions to be drawn from these results. First, since the total cost of electrodialysis is largely governed by individual contributions which are proportional to membrane area (especially membrane replacement, fixed capital investment and labor), this cost will be relatively insensitive to feed concentration. The second point is that this situation is a consequence of being voltage-limited. These results provided the impetus for estimating the effect of operating at the limiting current instead of at the limiting voltage in the event that this constraint could be relaxed. (Methods of increasing the limiting voltage include "deadening" the membranes around the manifold holes and rubber-coating the troublesome areas.)

In most electrodialysis systems, the limiting current is roughly proportional to the conductivity of the solution to be demineralized. Since the conductivity of the whey containing 18.24% total solids was about 1.7 times that of raw whey, the limiting currents should vary by about the same factor. The rate of demineralization should vary by approximately this factor as well. Consequently, much less membrane area would be required for a given degree of demineralization and the membrane replacement cost and costs related to membrane area would be much lower if concentrated whey feeds were used rather than raw whey. The cost of electrical energy increases substantially, but it is still minor compared to costs related to membrane area. Rough cost estimates were made and savings for whey containing 18.24% total solids were approximately 50% for the current limited case over the voltage limited case while savings for raw whey were only 20%. This indicates that the cost of electrodialysis should be significantly lower and more sensitive to feed concentration for the current limited case than for the voltage-limited case. This is due to the fact that, in the current limited case, full advantage is being taken of the solution's ability to transport charge and this ability increases with feed concentration.

Prefractionation by ultrafiltration

The experiments conducted on whey that had been prefractionated by ultrafiltration involved the demineralization of whey that had been reduced in volume by approximately 50%, 67%, 80% and 90% and the comparision of these results with the data obtained from demineralizing raw whey. The compositions of the various feeds are recorded in Table 4 and the demineralization curves are shown in Figure 8.

The important thing to notice about the curves is that even though the relative ash concentration increased by 27% over the range of volume reductions encountered (on a wet basis), the demineralization curves for the greater volume reductions (higher ash concentration) were below the demineralization curve for raw whey, a different situation than that encountered with feeds preconcentrated by reverse osmosis. This result indicated a distinctly lower rate of demineralization for the higher volume reductions as shown in Table 4.

The compositions of the feeds recorded in Table 4 are quite different, so the method of economic comparison used for feed preconcentrated by reverse osmosis is not very meaning-

Table 4—Data to determine the effect of protein concentration on demineralization

Volume reduction	0	50%	67%	80%	90%
Wt % ash (Wet basis)	0.655	0.647	0.691	0.785	0.856
Wt % protein (Wet basis)	0.72	1.28	1.80	3.34	6.59
Protein/ash	1.12	1.99	2.61	4.25	7.74
Conductivity (microhms/cm)	6300	6100	6200	6000	5500
Wt % ash removed for 50% demineralization	0.328	0.324	0.346	0.392	0.428
Time for 50% demineralization[a]	13	15	15.3	21.1	24.6
Relative rate[b]	1.00	0.855	0.892	0.738	0.692
Applied voltage (volts)	77	79	78	78	83
Energy consumed (Joules) X 10^{-5}	2.41	2.68	2.80	3.22	3.86

[a] Corrected to new membrane value
[b] Ratio of the rate of demineralization (weight % ash removed per minute) to the rate of demineralization for raw whey

ful here. The basis of economic comparison when the feed has been prefractionated by ultrafiltration should take into account the value of the product, which is established largely by its protein content. Before discussing the effect of prefractionation by ultrafiltration on the cost of production, it should be noted that in some cases demineralization of the feed may not be necessary. Two national manufacturers of whey products and whey derivatives market a product which contains approximately 50% lactose, 30% protein and 10% ash. It is produced by thermally concentrating the whey, crystallizing out lactose, and removing 50% of the ash by electrodialysis. An 80% volume reduction by ultrafiltration produces a product of the same proportions except that the ash concentration is only 7%, so that there is no electrodialytic demineralization required (see Table 2). In fact, all of the volume reductions quoted in Table 2 yield retentates with less than 10% ash in the dry solids. If the combined cost of thermal evaporation and lactose crystallization exceeds the cost of ultrafiltration to achieve the same total solids concentration and composition, then the latter approach could result in significant savings in the production of high protein whey products.

The demineralization brought about by ultrafiltration also opens the way for the production of products of varying protein content and very low ash content. We will briefly discuss the economics of producing such products with an ash content of four percent.

Since the amount of ash that had to be removed varied widely and the system was voltage-limited, the production rate on a dry weight basis also varied widely. The rate of ash removal was not as constant in this case as it was for feeds preconcentrated by reverse osmosis. The ash removal rate decreased significantly, a point that will be discussed further. However, the amount of ash that had to be removed decreased much more rapidly with feed concentration, so the solids production rate increased significantly, varying from 41 lb/day (raw whey) to 252 lb/day (90% volume reduction). This large increase in production rate for a given membrane area as the feed concentration increased implies that the cost of electrodialysis should decrease drastically for the more concentrated feeds. However, the prefractionation cost increases under these conditions. The result, according to rough economic estimates, is that the total production cost of dry whey solids containing 4% ash and varying concentrations of protein can be expected to be relatively uniform for a wide range of feed concentrations. Since the product value should go up as protein concentration increases, the profitability of the venture should increase with feed concentration.

Two factors which have been omitted from the discussion concerning prefractionation by ultrafiltration should be mentioned. First, no provision is made for the disposal of the permeate from the ultrafiltration process. This permeate has

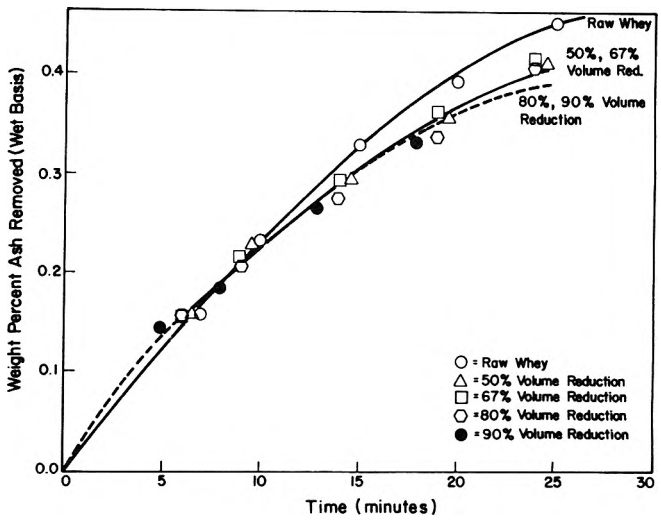

Fig. 8—Demineralization curves for whey prefractionated by ultrafiltration.

roughly the same lactose concentration as raw whey and thus represents a waste treatment problem. Concentration of the permeate by reverse osmosis followed by crystallization of lactose is a possible solution. Another suggested processing step is protein production via yeast fermentation. Second, we have not estimated the effect of operating at the limiting current in this system. It was felt that any estimate in this area would be too vague and speculative due to the widely varying protein concentrations in the various feeds.

Effect of protein concentration on the rate of demineralization

In addition to providing the data for a rough economic analysis of the production of whey products of varying protein content, the experiments on whey prefractionated by ultrafiltration also provided information necessary to determine the effect of protein concentration on the demineralization process. The data relevant to this discussion are found in Table 4 and Figure 8.

From the data, it seems obvious that the protein molecules hinder the mobility of the ionic species in the whey. Although the ash concentration increases by 27% over the range of concentration, the conductivity of the whey decreases by 12% over the same range. The conductivity decrease is accompanied by a sevenfold increase in the protein-to-ash ratio.

Table 5—Demineralization data for whey prefractionated by ultrafiltration

Volume reduction	0	50%	67%	80%	90%
Wt % ash (Wet basis)	0.655	0.647	0.691	0.785	0.856
Wt % ash that must be removed to produce whey solids containing 4% ash	0.438	0.391	0.400	0.357	0.231
Percent demineralization to produce whey solids containing 4% ash	67.0	60.5	57.8	45.5	27.0
Time to achieve required percent demineralization (min)[a]	20.6	21.8	20.6	18.5	10.4
Energy consumed (Joules) X 10^{+5}	3.2	3.14	2.99	2.86	1.90

[a] Based on 13 min for 50% demineralization of raw whey (new membrane value)

Table 6—Relative current efficiency factor, ξ

Feed	ξ
Raw whey	0.86
Raw whey (High temp)	1.00
50% Volume reduction (UF)	0.76
67% Volume reduction (UF)	0.77
67% Volume reduction (UF-high temp)	0.78
80% Volume reduction (UF)	0.76
90% Volume reduction (UF)	0.74
12.17% Total solids (RO)	0.82
18.24% Total solids (RO)	0.85

A second effect of an increased protein-to-ash ratio is a significant decrease in the rate of demineralization. The conductivity of the whey decreased by 12% over the range of concentrations studied and the applied voltage was increased by 8%, so a fairly constant rate of demineralization was expected. However, this rate dropped by 31%. Since the rate of demineralization is proportional to the product of voltage, stack conductivity and current efficiency, either a decrease in the current efficiency or a decrease in the stack conductivity beyond that expected could be responsible for this behavior. The additional decrease in conductivity could be attributed to different hydrodynamic conditions in the conductivity cell and the electrodialysis stack and the different effects that the protein molecules may have in each case.

Equation (3) provides a means of determining whether or not a decrease in current efficiency is involved in the lower rate of demineralization at high protein concentrations.

$$\frac{\text{Energy}}{\text{W.A.R.}} = \left[\int_0^t i\,dt\right] \frac{\text{Voltage}}{\text{W.A.R.}} \qquad (3)$$

where W.A.R. = Weight percent ash removed on a wet basis; i = current; and t = time.

Dividing both sides of the equation by the applied voltage will yield a term that is proportional to the reciprocal of the current efficiency. Since we know the energy consumed and the applied voltage, a "current efficiency factor" may be calculated. The results are tabulated in Table 6 and the generation of this table will be discussed shortly. The table reveals that the current efficiency factor varied significantly as the feed conditions were varied. This does not preclude an additional decrease in conductivity in the stack; in fact, current readings seemed to indicate this as well. However, our data did not permit a determination of the relationship between the two phenomena.

Current efficiency

In order to calculate the current efficiency, the total charge actually carried by the ash must be known, and this requires a knowledge of the ionic composition of the ash transported. If it is assumed that the composition of the ash transported in all runs was identical, a relative efficiency factor, ξ, may be calculated on the basis of the mass transported and the current required for that transport. This factor is tabulated in Table 6 for the range of feed conditions encountered in our experiments. It was calculated by dividing the weight percent of ash removed by the area under the current versus time curve in each case. The largest number was then arbitrarily assigned an efficiency value of 1.00, and the others were proportioned accordingly.

The value of ξ varies by approximately 25% over the range of feed conditions studied, the highest apparent efficiency occurring during the demineralization of raw whey at a high temperature, and the lowest occurring during the demineralization of whey containing the highest concentration of protein studied.

The apparent change in efficiency can be attributed to either a true change in efficiency or a change in the composition of the ash being transported (differing charge to mass ratio). Detailed studies of the ionic compositions of feed and product streams are currently in progress.

It was interesting to note that the current efficiency factor was quite independent of the condition of the membranes and the applied voltage in the runs involving raw whey. The composition and temperature of the fluid to be demineralized seemed to be much more important factors in determining current efficiency.

Effect of temperature

Experiments at high temperatures were very limited because membrane performance deteriorated rapidly under these conditions. The experiments conducted involved comparison of the performance of the system at 46°C to its performance at room temperature (approx 26°C) for raw whey and whey that had undergone a 67% volume reduction by ultrafiltration.

Since increasing the temperature of a substance increases its conductivity, the effect of increased temperature on the membrane area required for given degree of demineralization was similar to the effect of increasing the total solids concentration by means of reverse osmosis. Furthermore, the current efficiency seems to be increased somewhat at elevated temperatures (Table 6). A final factor to take into consideration is the influence of temperature on bacterial growth. In view of the importance of sanitation in the food industry, this factor should not be ignored. Any advantages to be gained must be weighed against the increased cost of membrane replacement at higher temperatures.

It is interesting to note that workers using the same type of Ionics cation membranes and a different type of Ionics anion membranes found little thermal degradation in demineralizing sea water at 65°C (McRae et al., 1968). The differences in the types of membranes used and the possibility of degradation being catalyzed by whey constituents should be investigated.

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Ms received 7/31/75; revised 2/13/76; accepted 2/16/76.

Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison and by the Stauffer Chemical Co.

The authors thank Ionics, Inc. and the Fluid Sciences Division of Universal Oil Products for making available membranes and equipment.

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CHEMICAL TREATMENTS OF COTTAGE CHEESE WHEY TO REDUCE FOULING OF ULTRAFILTRATION MEMBRANES

ABSTRACT

Increases in ultrafiltration rates were obtained by treating whey with acid, calcium-sequestering agents, or compounds to modify specific protein side chains, or by increasing the ionic strength. The proteins in whey, examined by scanning electron microscopy, underwent marked structural changes corresponding to these changes in the chemical environment. Depending upon the treatment, membrane fouling deposits showed strands, lattice networks, beaded matrixes, helical structures, or protein sheets of varying thickness. The protein forms were affected by the pH, the quantity of reagent added, and the protein functional group which was modified. Fouling of membranes was reduced when formation of protein sheets over the membrane was retarded.

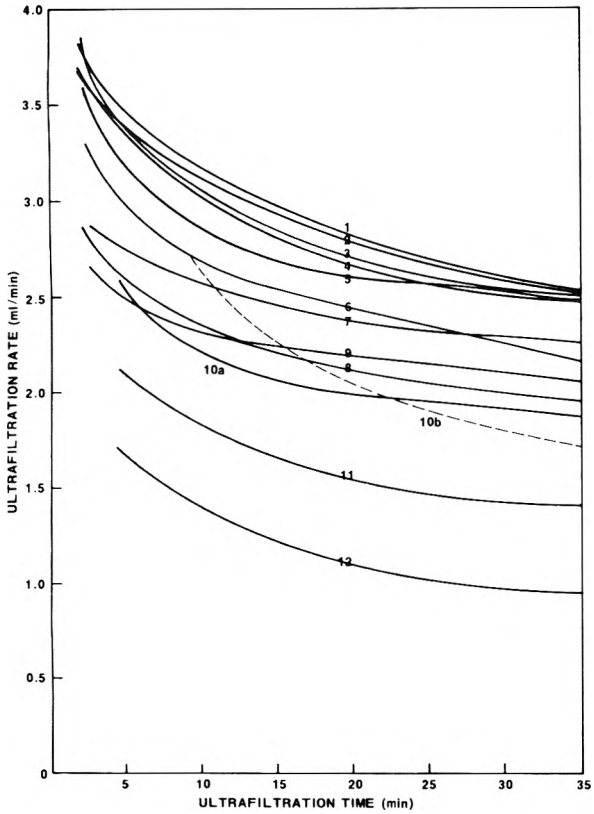


Fig. 1—Ultrafiltration rates for chemically treated cottage cheese whey: 1. 0.2M $CaCl_2$ and 1.5 X 10^{-3}M NEM added; 2. 0.2M $CaCl_2$ added; 3. 0.6M NaCl added; 4. 0.01M EDTA added; 5. 0.01M EDTA and 1.5 X 10^{-3}M NEM added, pH 5.5; 6. 1.5 X 10^{-3}M NEM added; 7. 0.2M sodium citrate added; 8. pH adjusted to 2.4; 9. 7.5M urea added; 10a. 7.5M urea, 1.33M glycine methyl ester, 0.1M EDC added; 10b. 7.5M urea, 1.33M GME, 0.1M EDC added (dialyzed against autoclaved whey); 11. untreated whey filtered through Whatman No. 1 paper (control); and 12. pH adjusted to 6.85 and centrifuged.

INTRODUCTION

WHEN MILK OR CHEESE whey is concentrated by reverse osmosis or ultrafiltration, deposits accumulate on the membrane to form a secondary membrane which reduces permeation rates and alters membrane selectivity. These deposits have been observed by electron microscopy by Glover and Brooker (1974) and by Lee et al. (1975a).

With cottage cheese whey, information on the fouling role of individual proteins (Lee and Merson, 1975; 1976a), coupled with SDS gel electrophoresis as an analytical tool (Lee et al., 1975b), has led to the development of pretreatments of whey to render it less fouling to membranes. Fourfold increases in ultrafiltration rate appear feasible by preultrafiltering the whey to remove high molecular weight proteins or protein complexes that contribute to a thick composite layer of fouling deposits (Lee and Merson, 1976a).

In the present study various chemical agents were used to promote permeation by dispersing or depolymerizing the whey proteins. The goal was to stabilize the suspension of fouling materials to prevent their deposition on the membrane. The general chemical environment of the proteins was altered by adjusting pH, or ionic strength, or by addition of urea. Specific protein interactions were induced or inhibited by the addition of calcium or calcium sequestering agents, or by the modification of sulfhydryl or carboxyl side chains.

Special attention was given to the possibility of β-lactoglobulin polymerization because conditions in whey lie within the range which favors octamerization (Timasheff and Townend, 1961; 1969). Furthermore, it had been observed earlier that both β-lactoglobulin and bovine serum albumin are sheet-forming constituents of whey (Lee and Merson, 1975). Thus, it was anticipated that blocking the sulfhydryl groups and carboxyl groups would help to disperse the whey proteins and retard the formation of the fouling layer.

For each treatment the ultrafiltration rate was determined and the nature of the fouling deposit was observed by scanning electron microscopy (SEM) and sodium dodecyl sulfate (SDS) disc gel electrophoresis.

THEORY

Changes in pH

It has been observed that acidification of whey increases ultrafiltration rates (Meggle, 1973; Hayes et al., 1974). This may be caused in part by pH induced changes in the state of β-lactoglobulin since this protein constitutes about 50% of the total protein in whey and the marked effect of pH on the association/dissociation behavior of β-lactoglobulin is well known (Timasheff, 1964; McKenzie, 1971; and Lee et al., 1975b). Dissociation to the monomeric form has been observed below pH 3.5. Between pH 3.5 and 5.4 association of β-lactoglobulin subunits to an octameric form is favored, especially at low temperatures. In the pH range 5.4–9.2, conformational changes of β-lactoglobulin are suspected to occur with a rapid ionization-linked transition taking place near 7.5 accompanied by increasing dissociation. Above pH 8, time-

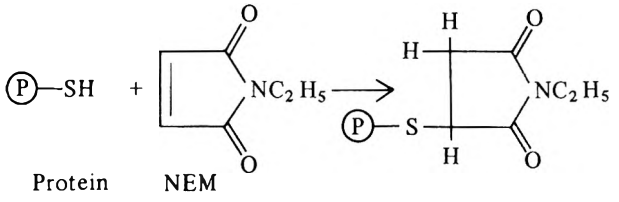
dependent aggregations occur slowly following the rapid transition. Thus, in the present study whey was further acidified with hydrochloric acid or neutralized with sodium hydroxide to ascertain the effect of pH on ultrafiltration rates.

Calcium addition and sequestration

Several investigators have suggested that residual casein contributes significantly to the fouling of membranes (Hayes et al., 1974; Lim et al., 1971). Therefore an attempt was made to clarify the role of casein indirectly by adding or removing calcium which in turn affects the stability of caseinate complexes. Either calcium or calcium-chelating agents (EDTA and sodium citrate) were added to whey to shift the equilibrium between colloidal calcium and ionic calcium. Thus, if casein is a significant fouling component, changes in the ultrafiltration rates should be observed corresponding to changes in the size and stability of the residual caseinate complexes.

Sulfhydryl group modification

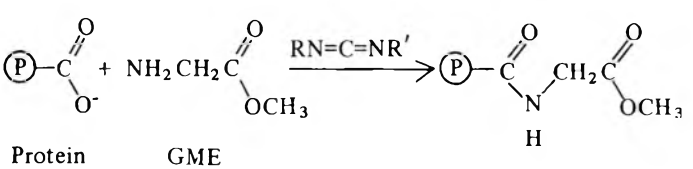
Two whey proteins contain sulfhydryl groups which may be active in association/dissociation phenomena. Bovine serum albumin contains 1.2 mole sulfhydryl per molecule (Boyer, 1954). The more abundant β-lactoglobulin contains two sulfhydryl groups per dimer (Beveridge et al., 1974) and furthermore these groups are situated relatively near the sites of intermolecular contact when the dimer associates to form the octamer (Townend et al., 1969). Roels and Lontie (1966) were able to stabilize β-lactoglobulin from disulfide interchange reactions by blocking the thiol groups using either ρ-mercuribenzoate or N-ethylmaleimide (NEM):



Thus NEM was added to whey before ultrafiltration to inhibit sulfhydryl group interactions.

Carboxyl-group modification

According to Timasheff and co-workers, carboxyl groups are involved in the self-association polymerization of β-lactoglobulin (Timasheff and Townend, 1969). The major association force is the formation of hydrogen bonds between the carboxyl groups of participating subunits. The resulting polymer structure is stabilized by hydrophobic interactions. Therefore, the procedure of Hoare and Koshland (1967) was used to chemically modify the carboxyl groups by conversion into amides through reaction with an amine, glycine methyl ester (GME) at pH 4.5–5. The reaction was promoted by the presence of a water-soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), and a denaturing agent (7.5M urea).



The procedure can also be used without the denaturing agent to selectively modify up to about half of the carboxyl groups. The carboxyl modification of whey proteins was conducted both in the presence and absence of 7.5M urea.

MATERIAL & METHODS

IN EACH ULTRAFILTRATION experiment, 250 ml of treated feed whey was ultrafiltered to one-fourth its original volume at room temperature using flat PM 10 membranes (Amicon) in a spiral-path flow cell (Amicon model CEC1) (Lee et al., 1975a). Both feed samples and retentate solutions were examined using a Cambridge Stereoscan Mark IIA scanning electron microscope operating at 10 kV acceleration voltage. The samples were mounted on Nuclepore membranes (Nuclepore Corp.) by passing 1 ml of solution through the 0.4 μm diam pore-size membranes. The samples were also analyzed using SDS disc gel electrophoresis (Weber and Osborn, 1969). Details regarding sample preparation and techniques involved in scanning electron microscopy (Lee et al., 1975a) and SDS gel electrophoresis (Lee et al., 1975b) have been reported previously. The retentate samples were obtained by disassembling the flow cell after 35 min of ultrafiltration and pipetting solution from the immediate vicinity of the undisturbed membrane (Lee et al., 1975a). These samples were called "immediate retentate" and contained fluid including loose deposits from very near the membrane.

Cottage cheese whey, cultured from pooled milk with mixed strains of lactic-acid-producing streptococci, was obtained commercially shortly after cheese manufacture. The whey was filtered through Whatman No. 1 filter paper and stored at 7°C until used (not over 7 days). All chemicals were reagent grade obtained commercially: Ethylenediaminetetraacetate (EDTA) (Matheson, Coleman and Bell), sodium citrate (Allied Chemical), urea, calcium chloride and sodium chloride (Mallinckrodt), N-ethylmaleimide (NEM) (Eastman Kodak), and glycine methyl ester (GME) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (Sigma).

RESULTS

REPRESENTATIVE ultrafiltration results for the pre-treated whey samples are given in Figure 1 and compared after 10 min of ultrafiltration in Table 1. All treatments produced increases in premeation rate except alkaline neutralization to pH 6.85 which caused a decrease in rate.

pH Adjustments

The reason for low permeation rates with the neutralized whey was that the alkaline treatment produced a thick, gelatinous, white material which completely filled the channels of the ultrafiltration apparatus. This caused a 52% decrease in permeation rate compared to untreated whey. Smaller decreases in permeation rate were obtained by pre-filtering the neutralized whey through Whatman No. 1 filter paper (−39%) or by removing the viscous material more efficiently be centrifuging for 25 min at 1500g and using the supernatant as feed (−25%). The latter result is plotted as curve 12 in Figure 1.

Figure 2 shows the typical deposit produced by untreated whey feed examined by SEM. Quite different thick granular deposits were observed with neutralized whey even after centrifugation (Fig. 3). The sample is immediate retentate redeposited on a Nuclepore membrane. Higher magnification (Fig. 4) showed the matrix nature of the deposits with characteristic fine strands evident where bacteria were present. As deposition proceeded the granular matrix was gradually covered over with layers of strands or finer deposits. Figure 5 shows a nearly completed sheet and also demonstrates the covering of pores by the fouling material (arrows).

In contrast to neutralized whey, whey acidified to pH 2.4 was a clear solution with no precipitate. Redeposited immediate retentate samples showed that acidified whey could still form sheets but the deposits were either much thinner (Fig. 6) or absent (Fig. 7). Consequently, permeation rates were about 30% higher than for the control whey.

Calcium sequestration

Calcium chelation was carried out by adding EDTA or sodium citrate to the whey. Permeation rate was increased 66% with 0.01M EDTA (Fig. 1, curve 4), but only 22% above the control whey when excess (0.2M) EDTA was added. Addition of 0.2M sodium citrate caused a 44% increase (curve 7).

Notably in the whey samples with calcium chelating agents

Table 1—Summary of whey treatments

Nature of treatment	Reagent added	pH Before ultrafiltration	Curve no. in Fig. 1	% Increase in UF rate after 10 min ultrafiltration
Cottage cheese whey (control)	–	4.3–4.6	11	–
pH adjustments	NaOH	6.85	12	–25%
	HCl	2.4	8	30%
Calcium sequestration	0.01M EDTA	4.1	4	66%
	excess EDTA (0.2M)	4.0	–	22%
	0.2M sodium citrate	6.1	7	44%
Increase in ionic strength	0.005M $CaCl_2$	4.3	–	40%
	0.2M $CaCl_2$	4.0	2	72%
	0.6M NaCl	4.2	3	69%
Breaking of competitive hydrogen bonds and minimizing hydrophobic interaction	7.5M urea	4.8	9	28%
Specific modifications				
a) sulfhydryl modification	1.5 X 10^{-3}M N-ethylmaleimide	4.4	6	49%
	3.0 X 10^{-3}M N-ethylmaleimide	5.0	–	49%
b) carboxyl modification	1.33M glycine methyl ester with 0.1M EDC			
	(i) in presence of 7.5M urea, urea not removed before UF	3.3	10a	20%
	(ii) in presence of 7.5M urea, urea removed before UF	4.5	10b	47%
	(iii) in absence of urea	2.9	–	16%
Combined treatments	1.5 X 10^{-3}M N-ethylmaleimide and 0.2M $CaCl_2$	3.9	1	76%
	1.5 X 10^{-3}M N-ethylmaleimide and 0.01M EDTA	4.1	–	40%
	1.5 X 10^{-3}M N-ethylmaleimide and 0.01M EDTA, pH adjusted	5.5	5	60%

added (Fig. 8–11), compared to untreated whey (Fig. 2), there was substantial decrease in the matrix-forming components (except for the microorganisms). One may speculate that this indicates a decrease in the size of residual caseinate or protein complexes so that they remained dispersed and passed through the Nuclepore pores.

With 0.01M EDTA added, fouling sheets of the immediate retentate appeared thinner and more fragile (Fig. 9) than those of the starting feed solution (Fig. 8), in contrast to untreated whey where immediate retentate deposits are much heavier than in feed samples. The pores were relatively free for water transport. Spiral structures similar to those observed in a study of β-lactoglobulin (Lee and Merson, 1976b) were present. At 0.2M EDTA in most micrographs the deposit was thicker, more uniform and not as lacy as in Figures 8 and 9. Under high magnification (Fig. 10) the coating, which occluded most pore openings, appeared to be composed of connected spherical particles about 300Å in diameter.

The 0.2M sodium citrate sample (Fig. 11) showed yeast cells (M.G. Miranda private communication, cf. Talens et al., 1973) and bacteria covered with a thin coating. The presence of yeast cells was confirmed by light microscopy. The coating between organisms was connected by fine strands and most pores were not covered over. It is believed that the microorganisms did not produce the stranding moieties but were merely functional in demonstrating their presence. The strands are believed to be the first stages of sheet formation.

Calcium addition

Heavy deposits were observed (Fig. 12) for whey with 0.005M $CaCl_2$ added. Microorganisms which presumably were deposited early (cf. Lee et al., 1975a, Fig. 2, 11, 12, 13) were completely covered by the thick film. The pair of microorganisms in the upper left-hand corner were deposited later and were covered by a light, stranded coating. It is postulated that these strands would fill in to form thicker sheets as time progressed. Another spiral structure, coated with deposits, was present.

Increasing the concentration of $CaCl_2$ to 0.2M caused a striking decrease in the amount of material composing the film (Fig. 13). The layer appeared brittle with numerous cracks. The layer covered the microorganisms but the membrane itself appeared clean.

Higher UF rates were obtained with whey containing the additional calcium chloride. The rates with 0.005M $CaCl_2$ and 0.2M $CaCl_2$ added were 40% and 72% higher than the control whey (Fig. 1, curve 2).

Because higher ultrafiltration rates were obtained by both sequestering and adding calcium, it was suspected that the main effect of calcium addition was to increase the ionic strength of the whey. Therefore, whey was adjusted to an ionic strength equivalent to the 0.2M $CaCl_2$ whey feed by adding 0.6M NaCl. Electron micrographs of this sample (Fig. 14) resembled those of the 0.2M $CaCl_2$ whey (Fig. 13) exhibiting a thin, incomplete sheet atop the large particles. Extensive stranding around the microorganisms and amorphous granules were evident in the enlarged micrograph, Figure 15. The 0.6M NaCl whey feed caused a 69% increase in permeation rate (Fig. 1, curve 3) compared to the 72% increase with 0.2M $CaCl_2$.

Sulfhydryl-group modification

Whey with either 1.5 X 10^{-3}M or 3.0 X 10^{-3}M NEM added ultrafiltered at the same rate, about 49% above untreated whey (Fig. 1, curve 6). The microorganisms present were coated with fine granular particles which show up as dark areas on the Nuclepore membrane in Figure 16. The partial sheet appeared to be smooth and stranding was not observed.

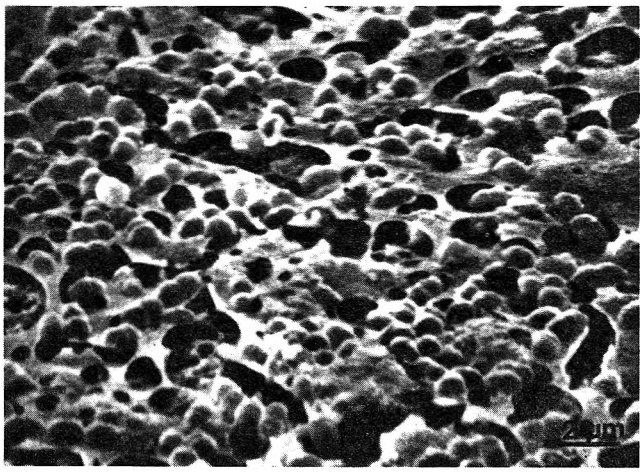


Fig. 2–Scanning electron micrograph of cottage cheese whey (control) mounted on a Nuclepore membrane (4,500X).

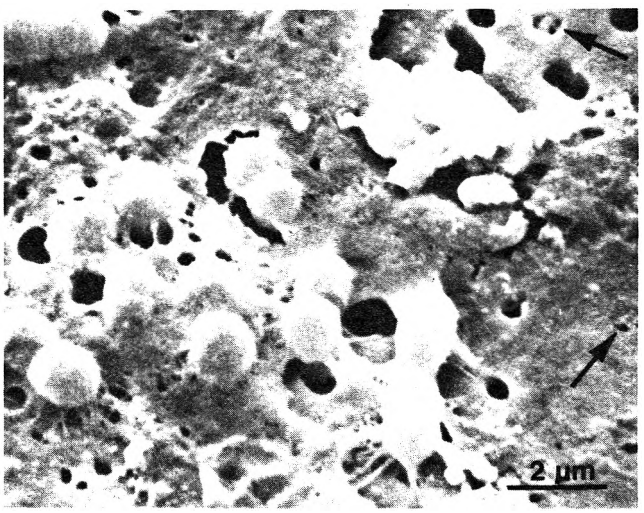


Fig. 5–Neutralized whey supernatant, pH 6.85 (6,000X). Arrows indicate partially clogged pores.

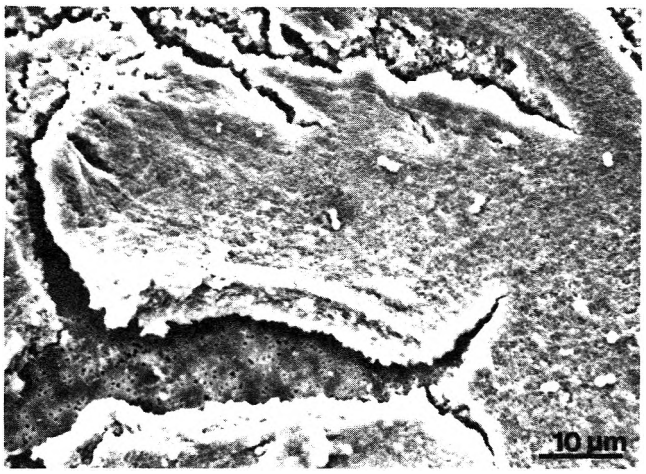


Fig. 3–Immediate retentate sample of neutralized whey supernatant, pH 6.85 (1,020X).

Fig. 6–Whey with pH adjusted to 2.4 (immediate retentate sample) (13,000X).

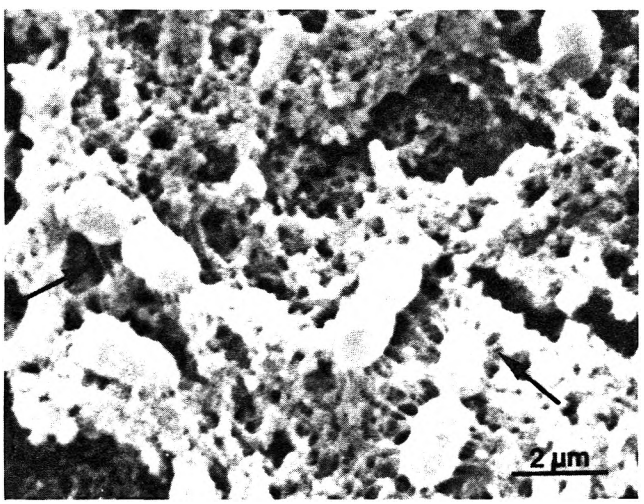


Fig. 4–Neutralized whey supernatant, pH 6.85 (6,000X). Arrows indicate strands extending over and around bacteria.

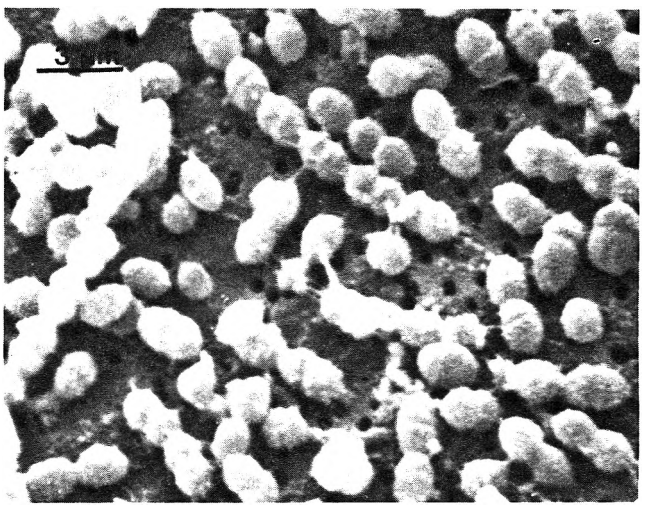


Fig. 7–A sheet-free region of the acidified whey sample (3,600X).

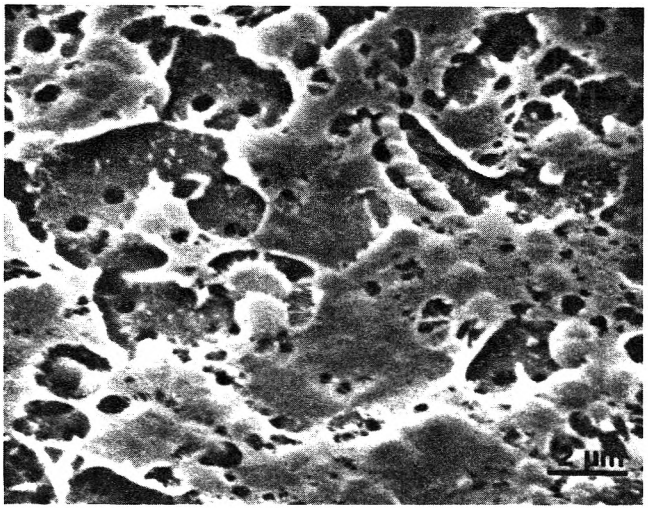


Fig. 8–Whey feed with 0.01M EDTA added (5,000X). Arrow indicates a spiral structure.

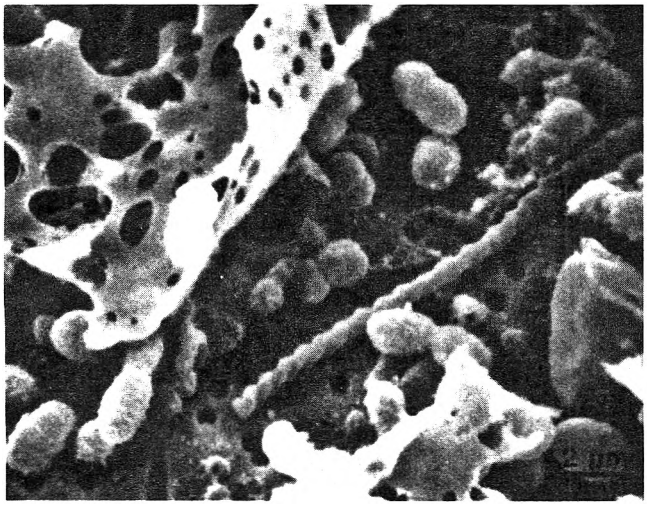


Fig. 9–Immediate retentate sample of the 0.01M EDTA whey (5,000X).

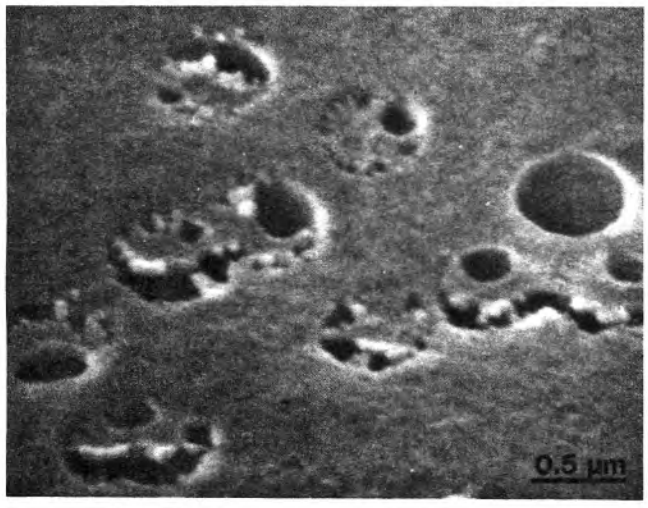


Fig. 10–High magnification of clogged pores in whey sample with 0.2M EDTA (excess) added, illustrating the particulate nature of the strands (24,000X).

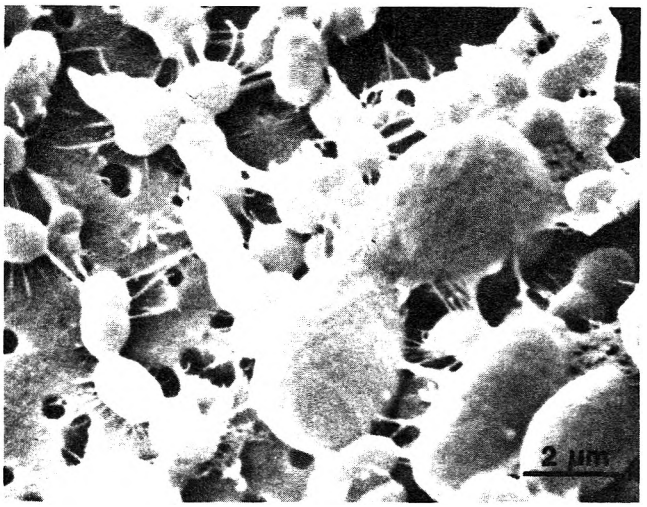


Fig. 11–Whey with 0.2M sodium citrate added (5,500X).

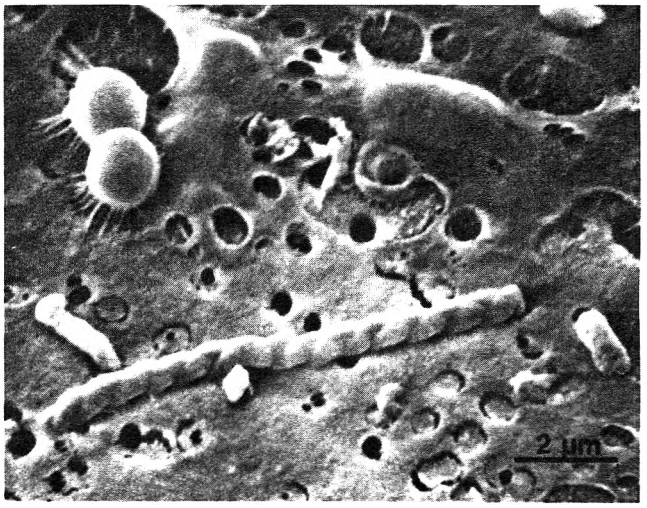


Fig. 12–Whey with 0.05M $CaCl_2$ added (6,500X).

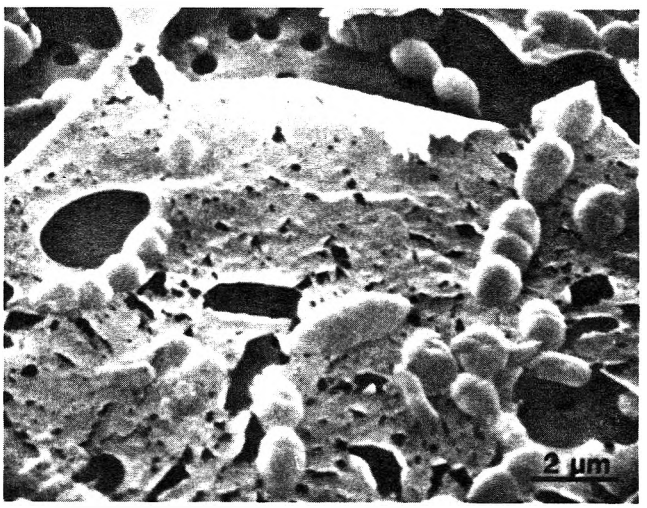


Fig. 13–Whey with 0.2M $CaCl_2$ added (5,500X).

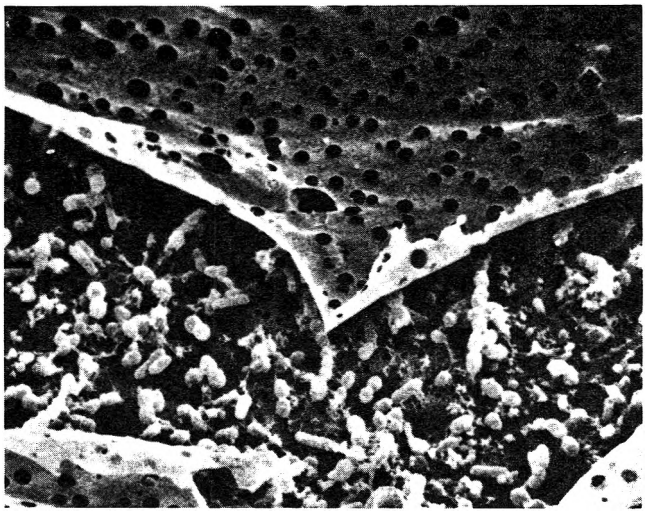


Fig. 14–Whey with 0.6M NaCl added (2,050X).

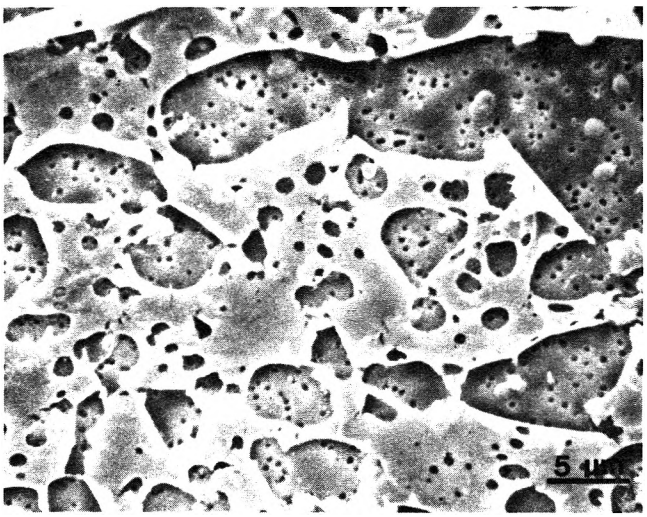


Fig. 16–Whey with 1.5×10^{-3} M N-ethylmaleimide added (2,200X).

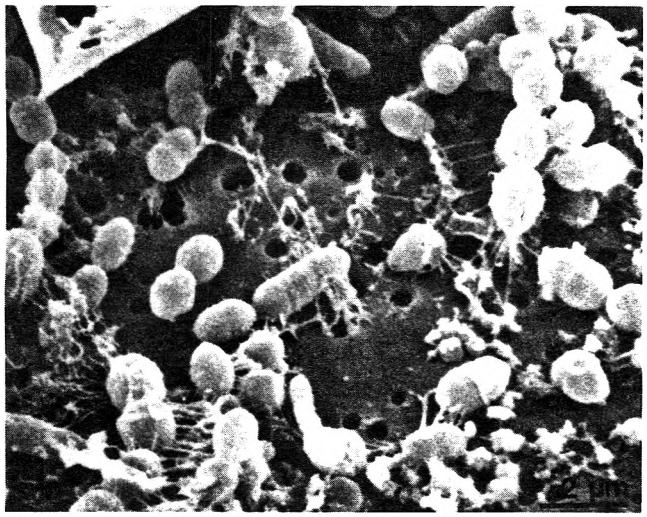


Fig. 15–Whey with 0.6M NaCl added (5,100X).

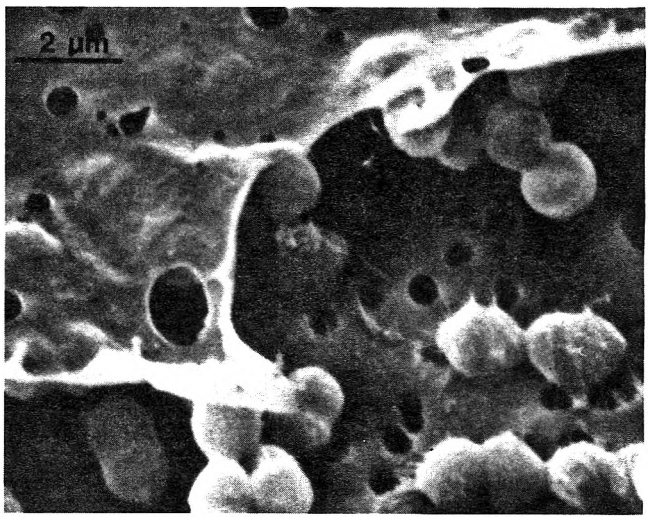


Fig. 17–Whey with 7.5M urea added (6,600X).

Carboxyl-group modification

Since the procedure to chemically modify carboxyl groups involved the use of concentrated urea, whey was first examined with only urea added. Addition of 7.5M urea alone caused a 28% increase in permeation (Fig. 1, curve 9). Again there was a sheet deposited above the microorganisms (Fig. 17). However the treatment produced finer strands than those in Figures 11 or 15. The microorganisms were coated with fine granular particles.

With the addition of glycine methyl ester (GME) in the presence of ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and 7.5M urea, the permeation rate was 20% higher than for unmodified whey (Fig. 1, curve 10a). However, the rate was lower than that of whey with just the urea added (curve 9). Without the addition of urea in the reaction media, carboxyl modification (presumably to a lesser extent) resulted in a 16% increase in permeation.

To try to separate the effect of urea from the effect of carboxyl modification, whey was treated by the method of Hoare and Koshland in the presence of 7.5M urea, and the urea was removed prior to ultrafiltration by dialysis at 7°C against several changes of deproteinized whey (autoclaved at 121°C). The pH of the final mixture was 4.5. The permeation rate (Fig. 1, curve 10b) after 10 min was 47% above that for untreated whey but the rate decreased much more rapidly than with whey or with other treated wheys. An electron micrograph for the immediate retentate after 35 min of ultrafiltration with this sample showed that the sheet-forming materials were present predominantly in a beaded, self-associating state (Fig. 18). Microorganisms were interspersed in the beaded matrix, which resembled that for the neutralized whey (Fig. 3, 4). However, the visible gelatinous precipitates which occurred in the neutralized whey were absent and this treatment appeared either to increase dispersion or to maintain sufficient porosity in the deposits to yield an improved permeation rate for at least a short time. However, the sharp decrease in rate indicates that the porous matrix was rapidly filling in with deposits.

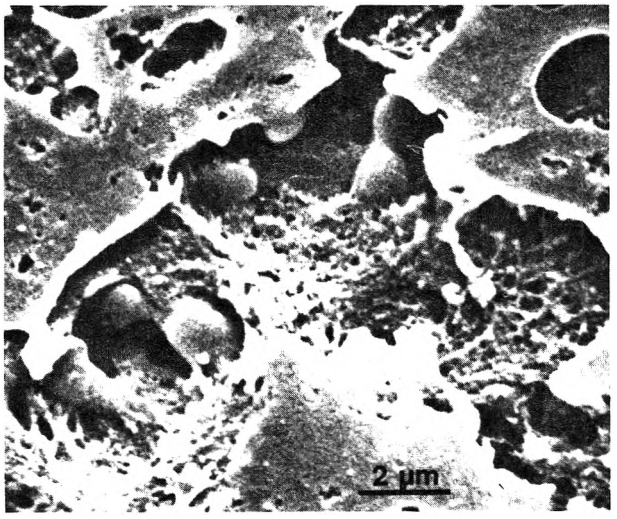


Fig. 18–Immediate retentate of whey with 7.5M urea, 1.33M glycine methylester, and 0.1M EDC added (5,500X).

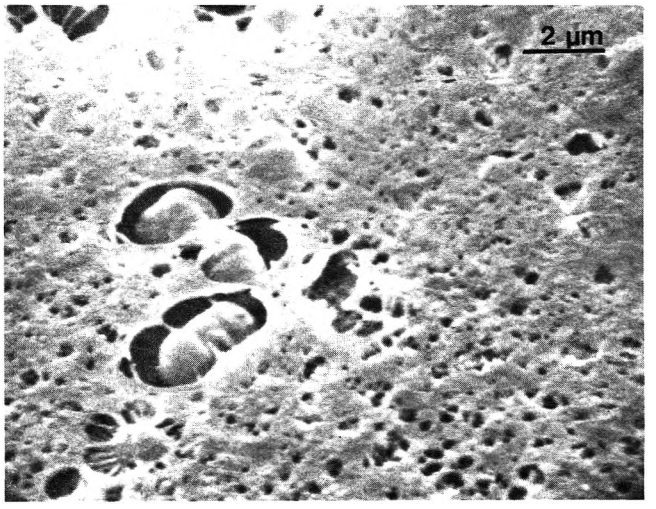


Fig. 20–Immediate retentate of feed whey with 0.2M $CaCl_2$ and 1.5 X 10^{-3}M NEM added (5,500X).

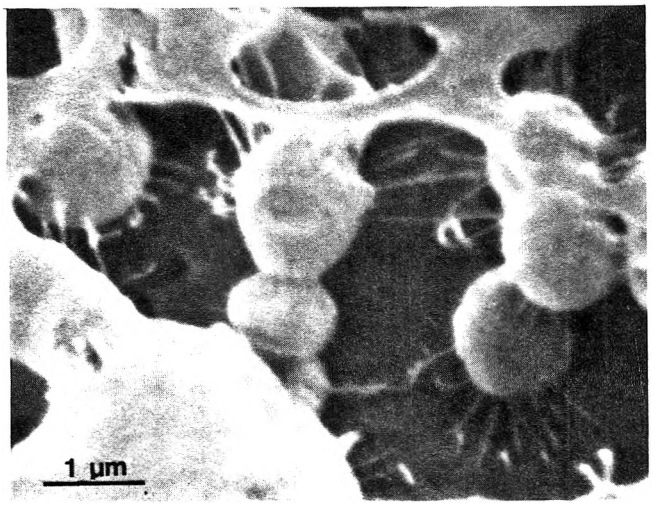


Fig. 19–Whey with 0.2M $CaCl_2$ and 1.5 X 10^{-3}M N-ethylmaleimide added (13,000X).

Combined modifications

Selected treatments which produced increases in permeation rate were tested simultaneously to determine if the separate improvements were additive. Whey with 0.2M $CaCl_2$ and 1.5 X 10^{-3}M NEM added resulted in a 76% increase in permeation (Fig. 1, curve 1). A 13,000X magnification of a representative region of the feed whey sample (Fig. 19) showed the microorganisms were stacked in layers. The sheet-forming components were laid down predominantly as strands being held up by the microorganisms. The pores were free of occluding deposits. The immediate retentate sample (Fig. 20) demonstrated the criss-crossed state of the overlapping stranding moieties. Presumably, this manner of deposition results in high porosity which permits a higher permeation than having the sheet-forming constituents associated into a completed sheet atop the ultrafiltration membrane.

Addition of 0.01M EDTA and 1.5 X 10^{-3}M NEM gave only a 40% increase in permeation. Adjustment of the final feed solution to pH 5.5 resulted in a 60% increase in permeation (Fig. 1, curve 5). However, this combined pre-treatment did not yield a rate higher than addition of 0.01M EDTA alone (curve 4). The sheet layer observed in Figure 21 with the immediate retentate sample for pH 5.5 was thinner than with pH not adjusted. Higher magnification (Fig. 22) of the indicated region in Figure 21 again reveals the criss-crossed pattern of buildup of the stranding moieties.

SDS disc gel electrophoresis

Electrophoresis revealed the presence of the β-lactoglobulin octamer (144,000 daltons) in addition to the regularly observed whey protein bands (Lee et al., 1975b) in the feed solutions of both the neutralized whey and the whey sample with both 0.2M $CaCl_2$ and 1.5 X 10^{-3}M NEM. Furthermore, octameric β-lactoglobulin and the BSA dimer (132,000 daltons) were present in retentate samples of all chemically pretreated wheys except those of the carboxyl-modified and acidified whey samples. Increased intensity of the octameric β-lactoglobulin band was observed particularly in samples that showed pronounced stranding features. No other significant changes in resolution of the whey proteins on SDS gels were detected and therefore gel patterns are omitted here.

DISCUSSION

DURING ULTRAFILTRATION the permeation rate will be high if the whey proteins are maintained in a dispersed state and not allowed to deposit on the membrane. If deposits do form, the rate will be maximized if the fouling structures are porous. Previous investigation of membrane fouling with cottage cheese whey (Lee and Merson, 1975; 1976a) has indicated that the relatively large independent particles, such as microorganisms and protein complexes, contribute to fouling by forming a matrix on the membrane. This matrix is porous but it stabilizes finer grained materials, notably β-lactoglobulin and BSA, which fill in the matrix and form sheets over it.

The addition of chemicals to whey alters the state of the whey proteins and changes the amount and structure of the fouling deposits. The abundant β-lactoglobulin is particularly susceptible to change, as indicated at the molecular level by

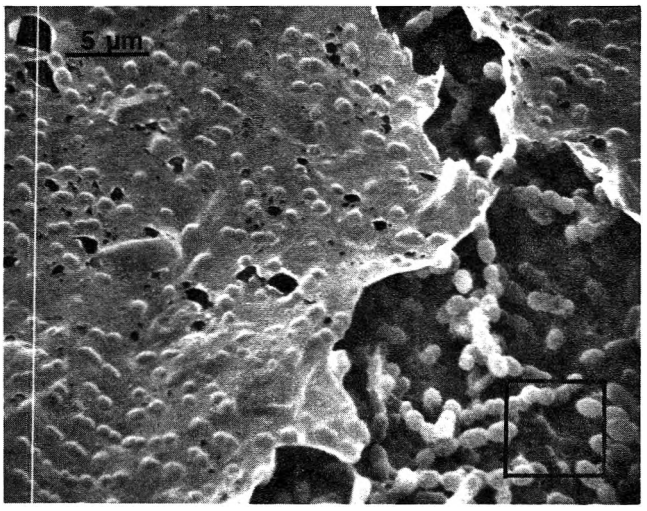


Fig. 21—Immediate retentate of feed whey with 0.01M EDTA and 1.5 X 10^{-3} M NEM added at adjusted pH of 5.5 (2,200X).

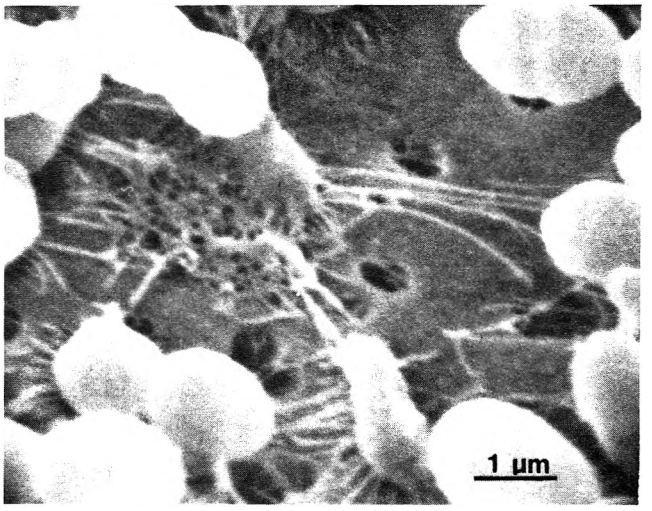


Fig. 22—High magnification of the indicated region in Fig. 21 (11,000X).

variations in the amount of octamer observed by electrophoresis. At the microscopic level, β-lactoglobulin exists variously as sheets, strands, fibers, helixes, or beaded structures depending on the treatment (Lee and Merson, 1976b). From the point of view of forming fouling deposits, both the sheets and beaded structures are undesirable. A treatment which improves permeation rate gives micrographs with only fine strands or stabilizes the proteins in solution resulting in clean microorgansims on the Nuclepore membrane.

In interpreting the micrographs, it is the deposits closest to the membrane which appear to have the greatest effect on permeation rate. The single, thin, incomplete sheet over the top of clean microorganisms and other large particles, as in Figures 6, 9, 13, 14, 17, 19 and 21, does not correspond to low ultrafiltration rates and apparently was formed during the last stages of sample preparation when the solution was filtered through the sample-mounting membrane to "dryness." Later experience confirmed that a procedure of (1) using more than 1 ml samples in the syringe, (2) passing only 1 ml through the sample-mounting membrane (as before) and (3) pouring off the excess sample, avoided formation of this film. Nevertheless, the thickness and apparent fragility of this induced sheet does indicate the effect of the chemical treatment on the whey constituents.

The chemical treatments may be divided into two classes: (1) a particular additive may affect the general solvent environment of the proteins, or (2) it may alter specific interaction sites. General modifications include adjustment of pH, altering ionic strength, or adding urea.

Figures 2 to 6 indicate that the form of the deposits is strongly dependent upon pH. Addition of NaOH to pH 6.85 destabilized the protein and caused thick deposits of a granular matrix which gradually filled in to form a nonporous fouling layer. Acidification to pH 2.4 stabilized the protein so that only light deposits were observed.

Increasing the ionic strength of the whey also altered the general environment. There was a significant thinning of the sheet layer as the amount of added $CaCl_2$ was increased from 0.005M to 0.2M. This implies that the dispersion of the protein in the whey was enhanced. The similar deposits and almost identical increase in permeation rate with NaCl at the same ionic strength supports the supposition that increasing ionic strength is the major effect here and not a specific interaction involving calcium. The mechanism is likely one of "salting in," the formation of an ionic sheath around the proteins which increases their solubility (see e.g., Von Hippel and Schleich, 1969). Much higher concentrations of salt, on the order of 3–4M for NaCl, could cause a decrease in the solubilizing power of the water around the proteins ("salting out") and one would expect increased membrane fouling, or even precipitates, under those conditions.

On the other hand, the specific equilibrium between colloidal and ionic calcium may be involved in the increased permeation rates caused by the chelating agents EDTA and sodium citrate. Removing some ionic calcium from the solution was expected to cause a reduction in the size of the residual calcium caseinate complexes and consequently a stabilization of these complexes (Jenness et al., 1966). However, too much calcium removal could have caused the complexes to reduce in size to the point of instability, consistent with the thicker deposits observed when the EDTA concentration was increased to 0.2M.

NEM addition was a specific treatment which markedly reduced the amount of fouling with only 1.5 X 10^{-3}M NEM. On the other hand, the modification of carboxyl group interactions with GME greatly altered the structure of the protein deposits but did not eliminate them nor improve the permeation rate very much. It should be pointed out that accurate interpretation of the increase in permeation rate for the NEM and GME modified wheys was hindered because the actual extent of chemical modification was not determined quantitatively. The changes in fouling were merely observed qualitatively.

It was hoped that combining treatments which produced increases in permeation rates by altering whey through different mechanisms would give substantial increases in rates. Combining the effect of increased ionic strength and reduced sulfhydryl-side-chain interaction did give a slight, but not important, additional improvement. Adding NEM to the 0.01M EDTA whey caused a small decrease instead. In general, the combined treatments studied were not effective.

From a commercial point of view, only addition of acid or a small amount of EDTA would be feasible to improve permeation rates when the proteins are to be recovered for food or feed uses. Salt and acid elimination are often objectives of ultrafiltration and NEM and GME would not be acceptable

food additives. They were employed in this study to assist in understanding the nature of the fouling deposits encountered during ultrafiltration of cottage cheese whey.

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Ms received 7/10/75; revised 1/31/76; accepted 2/4/76.

This research was supported in part by the Dairy Council of California.

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FIBROUS PROTEIN FROM COTTAGE CHEESE WHEY

ABSTRACT

A concentrate of whey protein (WPC) was prepared from cottage cheese whey by precipitation with sodium hexametaphosphate. Continuous fibers were spun by extruding a spinning solution into an acetic acid-sodium chloride coagulating bath. Spinning solutions consisted of WPC and sodium dodecylbenzene sulfonate dissolved in water at pH 9.0 containing 0.5% 2-mercaptoethanol. Residual detergent was extracted with aqueous acetone, and finished fibers were evaluated for diameter, tensile strength, elongation and rehydration properties. Stretching during formation reduced diameter and improved tensile strength. Single fibers and tows could be handled successfully and maintained their structural integrity during rehydration. Single-step extraction left less than 1% residual detergent.

INTRODUCTION

ONE OF THE PROBLEMS facing the cheese industry is the effective utilization of its major by-product, whey. While much progress has been made in the past few years, new uses for whey components will be needed to divert this material from an environmental pollution problem into new and useful food products.

Several proteins have been spun into edible fibers. Casein (Boyer, 1956), soy protein (Anson, 1957), peanut protein (Giddey, 1960), and single cell protein (Huang and Rha, 1972) are among those which have been used. This study was undertaken to ascertain whether whey protein could be used to produce a fibrous product.

EXPERIMENTAL

Whey protein concentrate (WPC)

A concentrate of whey protein was prepared from cottage cheese whey by the method of Hartman and Swanson (1966). Sodium hexametaphosphate with a mean chain length of 10 phosphates was used at a concentration of 0.5% and pH 3.0. The precipitated material was collected by centrifugation, washed with distilled water at pH 3, dispersed in distilled water, and freeze dried. The pH was adjusted with HCl. The dry material was ground to a powder and stored in sealed glass jars at room temperature until needed. Composition measurements included protein, ash, and moisture (AOAC, 1970); fat by the Babcock method for skim milk (Milk Industry Foundation, 1964); lactose (Hinton and Macara, 1927); phosphate as orthophosphate (ASTM, 1971).

Wet spinning apparatus

Figure 1 details the equipment used to spin whey protein fibers. Spinning solution was pumped through viton tubing, 0.08 cm i.d., with a Cole-Parmer Masterflex pump and extruded into the coagulating bath through a number 20 stainless steel hypodermic needle with a squared tip. Composition of the coagulating bath was 12% glacial acetic acid and 12% NaCl in distilled water. A circulating pump served to move the bath solution away from the extrusion point. At the distal end of the bath, the coagulated fiber was lifted out and onto a take-up reel which served to stretch the fibers. Unstretched fibers were cut and lifted out of the bath by hand instead of onto the reel. Hand removal of the fibers resulted in a small degree of stretching which was not measured. Stretched fibers were air dried on the reel and unstretched fibers were air dried on racks made from glass tubing.

Spinning solutions

Spinning solutions containing the detergent, sodium dodecylbenzene sulfonate, along with WPC and 2-mercaptoethanol resulted in fibers with desirable physical properties. Preliminary experiments showed that 1:1 ratios of WPC and detergent in the concentration range of 20–30% total solids, 0.5% 2-mercaptoethanol, and a pH of 9.0–11.0 (adjusted with 50% NaOH) gave workable fibers. Viscosity of the spinning solutions decreased with time, so three holding times (0, 6, and 24 hours after preparation) were used. The data reported are for WPC and detergent concentrations of 12 and 14% each, 0.5% 2-mercaptoethanol, pH 9.0, three holding times before spinning and stretched vs unstretched fibers.

Spinning solutions were prepared as follows. The desired amount of detergent was weighed and dissolved in distilled water then a weighed amount of WPC was added and dispersed by mixing with a magnetic stirrer. The 2-mercaptoethanol was added next, the pH was adjusted to 9.0 with 50% NaOH and the solution was made to volume with distilled water. The pH was checked and readjusted if necessary by adding a small volume of 50% NaOH. Preparation of the spinning solutions required about 30 min, and this constituted the 0 hr sample. Portions were held for 6 and 24 hr at room temperature for the other two time variables. Apparent viscosities of the various spinning solutions were measured with a Brookfield LVT viscometer at room temperature, 23°C.

Extraction of detergent

Residual detergent was extracted from air dried fibers with 60% aqueous acetone as suggested by Lundgren (1945). Acetone solutions with and without 5% KCl added were used. Sets of individual fibers, stretched and unstretched, and tows made of bundles of 100 stretched fibers were placed in Pyrex glass dishes, 26 × 16 × 4 cm, and covered with 500 ml of extracting medium. The dishes were covered with parafilm and extraction time of 24 hr for fibers and 48 hr for tows at room temperature were used. After extraction, fibers and tows were soaked in distilled water for 20 min, removed, and air dried.

Physical properties

Tensile strength and percent elongation of finished fibers were measured with an Instron Model 1782 universal testing machine which

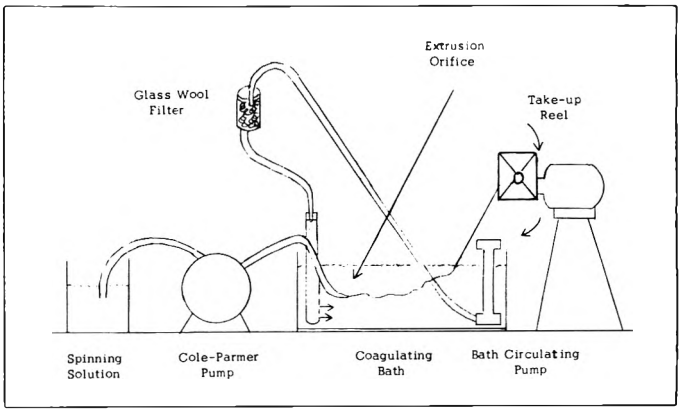

Fig. 1—Fiber spinning apparatus.

was calibrated before each use. Fibers and tows were conditioned in the Instron testing room for 48 hr at 70% relative humidity and 25°C before tests were made. Ten pieces of single fibers 5 cm long were chosen randomly from each of two replicate treatment lots. The pieces were cut, and 10 observations of fiber diameter were made microscopically with an eyepiece micrometer on one portion. The remaining portion of each fiber was cut into two pieces, and each piece was tested on the Instron. Operating conditions were:

Tension load cells:	50g for fibers, 500g for tows
Full scale load:	10g for fibers, 100g for tows
Gage length:	5.08 cm
Crosshead speed:	25.4 cm/min
Chart speed:	25.4 cm/min
Elongation:	20%/cm chart travel

Tensile strength and elongation were calculated from the stress-strain curves obtained.

Composition of finished fibers and tows

Finished fibers and tows were dried to constant weight in a vacuum oven at 60°C then analyzed for protein, phosphate and detergent. Samples of 100 mg of dry fiber material were weighed into 10 ml volumetric flasks, 5 ml of 0.05% NaCl was added to wet the sample, and they were made to volume with 2N NaOH. Stock solutions of fiber dispersions so prepared were analyzed for protein by a biuret method (Interdepartmental Committee on Nutrition for National Defense, 1963). WPC of known protein content determined by the Kjeldahl method was used as the standard protein for generating a regression equation for protein.

The equation was:

$$Y = -0.0059 + 0.061X$$

where Y and X were absorbance at 540 nm and weight of protein, respectively. The correlation coefficient was 0.996. Phosphate content was determined by the same method used for phosphate in WPC. Wisherchen's (1967) method was used to determine residual sodium dodecylbenzene sulfonate.

Statistical methods

The data were subjected to analysis of variance. Data dealing with viscosity of spinning solutions, composition, and physical properties of finished fibers were analyzed with a split plot design, and differences among means were analyzed by Tukey's W-procedure (Steel and Torrie, 1960).

RESULTS & DISCUSSION

Whey protein concentrate (WPC)

The composition of WPC made from cottage cheese whey is given in Table 1. Total protein recovery averaged 59.5% from whey containing a mean concentration of 0.88% protein. The failure of the composition data to total 100% suggested that some 7.4% of the material consisted of protein-bound water which was not removed by the vacuum oven method used for moisture determinations.

Fiber spinning

The addition of a detergent to WPC spinning solutions introduced an undesired additive which had to be removed later in the process, so a variety of methods were employed in attempts to form fibers directly from WPC. Dry WPC was dispersed in NaOH solutions ranging from 0.15–2.5M with WPC concentrations of 1–15% in 1% increments. In separate experiments, 5, 10, or 20% WPC was dispersed in water at pH values from 7.0–12.0 in 1.0 pH unit increments adjusted with NaOH. These treatments were repeated with 0.5, 1.0, and 2.0% 2-mercaptoethanol added to the WPC dispersions. None of these conditions produced fibers with satisfactory physical properties.

Fibers with "poor physical properties" were those which could not be lifted out of the bath without breaking or which could not be stretched. Microscopic observation of stretching these fibers as the spinning solution was extruded into the coagulating solution revealed that a thin, almost brittle surface layer coagulated quickly while the interior of the fiber remained fluid. As the fiber was pulled out, the surface layer broke, and succeeding surface layers coagulated resulting in a fiber with progressively decreasing diameter. When fibers were extruded into the coagulating bath and allowed to remain until they did completely coagulate, the fibers were very brittle and could not be manipulated. Although none of the treatments without detergent gave satisfactory fibers, the spinning solution which most nearly approached success was 15% WPC, pH 9.0, and 0.5% 2-mercaptoethanol. Concentrations of WPC above 15% and pH values in excess of 9.0 resulted in gelation of the dispersions.

Table 1—Composition of WPC made from cottage cheese whey

Component	Percent[a]
Protein	70.8
Lactose	2.7
Fat	3.1
Ash	11.4
Moisture	4.6
Phosphate	4.3

[a] Means of three lots sampled four times

Lundgren (1949) investigated the formation of protein fibers with spinning solutions containing egg albumin and sodium dodecylbenzene sulfonate. Spinning solutions having detergent to protein ratios of 0.66 to 1.5 and 18 to 24% total solids resulted in optimum fibers. The ability of reduced protein to bind more detergent than its native counterpart (Hunter and McDuffie, 1959; Pitt-Rivers and Impiombato, 1968), together with increased solubilization of protein by detergents contributed to improved fibers when 2-mercaptoethanol was added to the spinning solutions.

In agreement with Lundgren's (1949) findings, fibers with good physical properties were obtained with spinning solutions containing 25–30% total solids, sodium dodecylbenzene sulfonate to WPC ratios of 1.0 to 1.5 in the presence of 0.5% 2-mercaptoethanol. At pH values above 9.0 there was no additional beneficial effect on observed fiber properties, so pH 9.0 was chosen for all further work. Lundgren (1945) obtained improved fibers when spinning solutions were aged after preparation. His results, plus the fact that WPC–detergent dispersions underwent viscosity changes over time, indicated that this parameter should be included.

Evaluation of fibers

Table 2 shows the effect of the variables employed on fiber diameter. Stretching, as expected, had the greatest effect on this parameter. Analysis of variance by the split plot design indicated that fiber diameter was not significantly affected by WPC and detergent concentrations or by time elapsed before spinning. However, comparison of means by Tukey's W-procedure (Steel and Torrie, 1960) showed an inverse effect on diameter by concentration of WPC and detergent. Some differences were also significant for time and extracting solution.

Analysis of variance and comparison of means of the Instron data for tensile strength, Table 3, indicated that the only significant effect was from stretching the fibers which increased tensile strength. Conversely, elongation of stretched fibers was less than that of unstretched fibers, Table 4. The magnitude of this effect was not large as indicated by the values of the means. These effects might be explained by orientation phenomena brought about by stretching. Stretching should induce longitudinal orientation of unfolded protein molecules while the coagulated protein-detergent complex is still in a plastic state. Upon drying, the stretched, highly oriented fibers would be expected to withstand less elongation

Table 2–Diameter of finished fibers

Fiber type	Detergent extractant	Conc of detergent & WPC (%)	Age of spinning solutions 0 hr	6 hr	24 hr
			diam (cm X 10^4)[a,b,c]		
Stretched	60% Acetone	12	80.7a	71.8b	82.8a
		14	52.8d	54.0d	41.0e
	60% Acetone	12	79.0ac	66.3f	74.3bc
	+ 5% KCl	14	45.4e	43.4e	41.3e
Unstretched	60% Acetone	12	174.9g	181.1h	204.2k
		14	170.8g	162.9m	115.4n
	60% Acetone	12	184.7hj	171.0g	189.8j
	+ 5% KCl	14	130.7p	144.3q	111.3n

[a] Means of 10 observations on 10 samples from each of two replicate lots
[b] Means followed by the same letter are not significantly different at the 5% level by Tukey's W-procedure
[c] Standard error = 1.010

Table 3–Tensile strength of finished fibers

Fiber type	Detergent extractant	Conc of detergent & WPC (%)	Age of spinning solutions 0 hr	6 hr	24 hr
			Tensile strength[a,b,c] g/cm^2		
Stretched	60% Acetone	12	25900a	27100a	19300a
		14	23200a	30300a	38100a
	60% Acetone	12	18700a	29000a	19500a
	+ 5% KCl	14	25200a	18900a	25500a
Untretched	60% Acetone	12	8400b	8300b	7500b
		14	9200b	9800b	8800b
	60% Acetone	12	8200b	8500b	10500b
	+ 5% KCl	14	9100b	7200b	9600b

[a] Means of two measurements on 10 fibers from each of two replicate lots
[b] Means followed by the same letter are not significantly different at the 5% level by Tukey's W-procedure
[c] Standard error = 515.26

Table 4–Elongation of finished fibers

Fiber type	Detergent extractant	Conc of detergent & WPC (%)	Age of spinning solutions 0 hr	6 hr	24 hr
			Elongation, %[a,b,c]		
Stretched	60% Acetone	12	1.80a	1.51a	1.63a
		14	1.79a	1.65a	1.56a
	60% Acetone	12	1.50a	1.78a	1.68a
	+ 5% KCl	14	1.55a	1.47a	1.44a
Unstretched	60% Acetone	12	2.95b	2.90b	2.34b
		14	2.68b	2.90b	2.35b
	60% Acetone	12	3.28b	2.83b	3.15b
	+ 5% KCl	14	2.15b	2.30b	2.95b

[a] Means of two measurements on 10 fibers from each of two replicate lots
[b] Means followed by the same letter are not significantly different at the 5% level by Tukey's W-procedure
[c] Standard error = 0.391

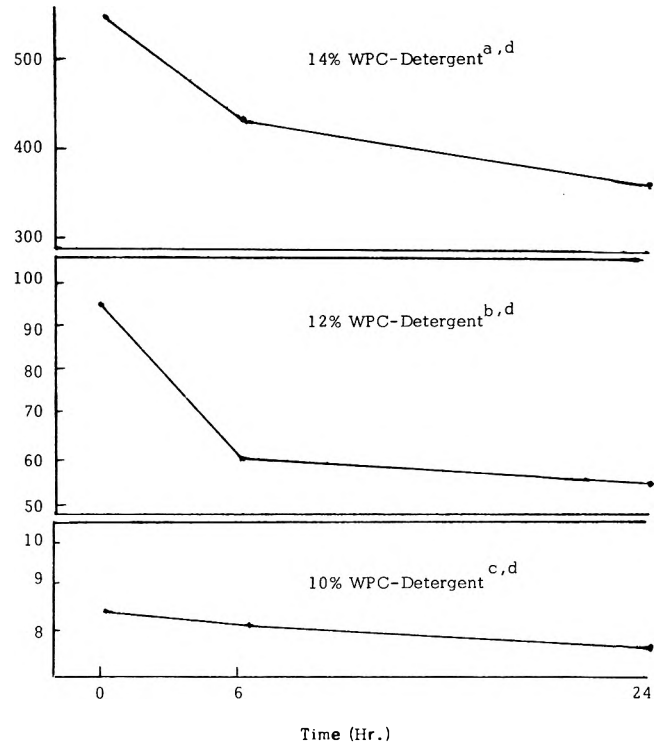

Fig. 2–Changes in apparent viscosity of spinning solutions as a function of time. ([a]Standard error = 38.14; [b]Standard error = 0.40; [c]Standard error = 0.14; [d]Means of three replicate lots, each sampled in triplicate.)

before breaking than fibers which had been prepared so that less orientation resulted, i.e., without stretching. Work by Moncrieff (1970) supports these assumptions. Similarly, a fiber consisting of longitudinally oriented protein molecules could be expected to have greater tensile strength than one consisting of protein molecules in a more random arrangement after the fibers had been air dried.

Apparent viscosity relationships

A change was noted in apparent viscosity of spinning solutions over time. Measurements were made on WPC dispersions, detergent solutions, and mixtures of the two made into spinning solutions. At zero time, 10, 12 and 14% WPC dispersed in water at pH 9.0 with 0.5% 2-mercaptoethanol showed apparent viscosities of 4, 4.8 and 5.5 centipoise. The same concentrations of the detergent, sodium dodecylbenzene sulfonate, showed apparent viscosities of 2.5, 3.9 and 3.4 centipoise. There was no apparent change over a 24-hr period, and the shearing action involved in making the measurements with the Brookfield instrument had no effect on measured values. However, when WPC and detergent were mixed to prepare spinning solutions, there was an effect from time of aging and a profound effect from concentration of WPC and detergent. Figure 2 demonstrates these changes. Analysis of variance of the data indicated significant effects ($P < 0.001$) from concentrations, elapsed time, and their interaction to produce a non-linear decrease in apparent viscosity with time, and much higher variation among the 3 replicates were noted at the 14% level. In spite of the variations noted, no consistent effect of apparent viscosity of spinning solutions on the physical properties of finished fibers can be noted from time effects in Tables 2, 3, and 4.

Composition

The protein, detergent, and phosphate contents of finished fibers and tows are shown in Table 5 on a dry weight basis. The protein content was greater than 93% in all samples tested, and ranged higher in fibers and tows prepared with 12% WPC and detergent than in those made from 14%. In general, the phosphate content of fibers and tows was lower when they were prepared from spinning solutions of the lower concentration. The concentration of neither of these components appeared to be affected by stretching nor by the solution used to extract the detergent. Moisture content was consistently $< 5\%$.

Comment

The amount of detergent that was extracted by the one-step extraction process was of concern. When the data on residual detergent in Table 5 were analyzed by analysis of variance, none of the treatments were significant. It was possible to produce finished fibers and tows consistently with less than 1% residual detergent with individual samples as low as 0.30% by the single extraction procedure. Sodium dodecylbenzene sulfonate is not accepted as a direct food additive, though it has been approved for fruit and vegetable wash solutions (Anon., 1965), in the preparation of plastics for food containers (Anon., 1966; Anon., 1970) and at unspecified levels for lye peeling solutions (Rothchild, 1973). Human volunteers given 0.1g doses of alkyl benzene sulfonates daily for four months showed no ill effects (Anon., 1967). Considering that dry fibers after spinning would contain almost 50% detergent, the one-step extraction procedure with 60% aqueous acetone was an efficient way to remove the bulk of the detergent. However, if the finished fibers were used in food application, the residual detergent should be further reduced by sequential extraction, or a protein dispersing agent which is acceptable as a direct food additive should be employed.

For these materials to have food applications, particularly as meat analogues, they should retain their fibrous integrity through a reasonable cooking treatment, knit well as bundles, have a bland flavor and acceptable mouthfeel. Dry finished fibers, when subjected to boiling in water for 30 min, retained their fibrous character and absorbed water to the extent of 1.25 times their dry weight. Although the dry fibers and tows were rather brittle, they quickly absorbed water at room temperature and became pliable. Rehydrated fibers were bland in flavor and had a pleasing mouthfeel, somewhat resembling small fiber bundles of cooked chicken meat. Tows made by collecting 100 stretched fibers as a bundle on the take-up reel behaved similarly when rehydrated and heated in water, and the tows did not separate into individual fibers. This indicated that no additional binder would be needed.

The results indicated that protein fibers could be produced from aqueous WPC dispersions, but that the physical properties of such fibers were so poor that they were of no practical value. Twelve and 14% dispersions of WPC containing equal concentrations of the detergent, sodium dodecylbenzene sulfonate, in the presence of 0.5% 2-mercaptoethanol at pH 9.0 formed continuous fibers which could be stretched and made into tows. The bulk of the residual detergent could be extracted with 60% aqueous acetone. The finished fibers and tows retained their fibrous integrity through 30 min boiling in water. Overall mean composition of the finished fibers was 98.5% protein, 0.6% detergent, and 1.0% phosphate on a dry weight basis.

Table 5—Compositions of finished fibers and tows (dry wt basis)

Fiber type	Conc of detergent & WPC	Protein[a]	Phosphate[b]	Detergent[a]
		%[c]		
Stretched	12	99.1	0.32	0.60
	14	96.5	1.39	0.54
Unstretched	12	98.1	0.39	0.69
	14	95.4	1.55	0.61
Tows	12	103.1	1.21	0.56
	14	98.8	1.35	0.85

[a] Means of 12 samples from each of two replicate lots
[b] Means of six samples from each of two replicate lots, as PO_4
[c] Standard errors: Protein—0.629; Phosphate—0.025; Detergent—0.068

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Ms received 10/18/75; revised 1/3/76; accepted 1/8/76.

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A COMPARISON OF SOME CHEMICAL PROPERTIES OF YOGURTS MADE FROM CONTROL AND LACTASE-TREATED MILKS

ABSTRACT

Certain properties of yogurts made from regular commercial skim milk and from milk which had been treated with lactase enzyme so that 70–75% of the lactose was hydrolyzed to glucose and galactose were evaluated. Initially faster rates of acid production in lactase-hydrolyzed milks (LH) during fermentation resulted in somewhat shortened processing times, although a decrease in the rate of acid production occurred earlier during fermentation in the LH milk so that final product pH was similar for both the control and LH yogurts. Control yogurts contained 5.0% lactose and 0.2% galactose while LH yogurts contained 1.6% glucose, 1.5% lactose and 2.1% galactose. Appreciably more lactic acid was produced in LH yogurts and this effect has been related to fermentation by the starter culture organisms of a greater proportion of the available sugar as glucose.

INTRODUCTION

RECENT INVESTIGATIONS have shown that a significant proportion of the world's population is unable to digest the disaccharide lactose due to a deficiency of lactase in the intestinal mucosa. According to a statement released by the Food and Nutrition Board of the National Academy of Sciences in 1972, 60–90% of non-Caucasian peoples have low lactase activity. The inability to digest lactose may result in the manifestation of symptoms such as stomach cramps, flatulence and diarrhea when milk and certain other dairy products are consumed (McCracken, 1971). In a survey of the etiology of lactose intolerance, McCracken (1971) noted that fermentation breaks down the lactose in milk and, although the lactose never completely disappears, the proportion of lactose in a fermented product may be reduced enough to make a product compatible to intolerant people. However, commercial yogurts made in the United States have been found to contain appreciable amounts of lactose due to the practice of fortifying the yogurt mix with nonfat milk solids. Goodenough (1975) reported lactose values for commercial yogurts ranging from 3.3–5.75%. Even higher values have been reported elsewhere in the literature (Acott and Labuza, 1972). Thompson and Gyuricsek (1974) developed a process for the manufacture of a yogurt product low in lactose. Their procedure involves pretreatment of milk with lactase in order to convert the milk lactose to glucose and galactose. They reported that yogurt manufactured by their process set more rapidly than yogurt made by conventional methods and had good acceptability. This study was undertaken to define the microbial and chemical properties of yogurts derived from lactase-hydrolyzed milks and to gather information concerning the types of changes which might occur in other cultured dairy products manufactured from lactase-treated milks.

MATERIALS & METHODS

Bacterial cultures

The culture employed for yogurt manufacture was a mixed yogurt starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* ($LBST_4$) supplied by the Marshall Div. of Miles Laboratories, Madison, Wisc. in cryogenic form. Cryogenic cultures which were kept stored in liquid nitrogen were thawed and then inoculated into sterile skim milk. Transfers in sterile skim milk were made daily.

Preparation of hydrolyzed lactose milk

Skim milk fortified 4% w/v with nonfat milk solids was incubated with 300 μg/ml of *Saccharomyces lactis* β-galactosidase ("Maxilact," Enzyme Development Corp., N.Y.) to hydrolyze the lactose to glucose and galactose. Incubation was overnight at 4°C with constant stirring and resulted in hydrolysis of 70–75% of the lactose.

Preparation of yogurts

Fortified control and lactase-hydrolyzed (LH) milks were heat treated at 105°C for 15 min and then cooled to 43°C prior to inoculation. The milks were inoculated at a level of 2% with the mixed yogurt starter culture which had previously been grown at least twice for 24 hr at 30°C in sterile skim milk. The inoculated milks were incubated in a water bath at 44°C and acidity in the milks was allowed to develop until the pH decreased to 4.6. Finished yogurt products were refrigerated at 4°C.

Enumeration of starter culture organisms

Enumeration of the starter culture organisms was made by plate count using Hansen's Yogurt Agar (Porubcan and Sellars, 1973) prepared as follows: 15.0g Bacto-agar (Difco), 1.0g beef extract and 10.0g proteose peptone #3 (Difco) were dissolved in 900 ml of distilled water with boiling and then autoclaved at 121°C for 15 min. To the sterile agar cooled to 46–47°C was added 2.5g glucose, 2.5g galactose and 5.0g lactose per liter in the form of sterile 10% solutions of the sugars sterilized by Millipore filtration using a 0.22μ pore size Millipore filter (Millipore Corp., Bedford, Mass.). Pour plates were incubated at 37°C for 48–72 hr and then counted. The starter culture organisms were easily differentiated from each other on this medium since *L. bulgaricus* formed diffuse low mass colonies while *S. thermophilus* formed discrete high mass colonies. Identity of the two types of colonies was confirmed by microscopic examination.

Acid development

Acid development was followed by measuring pH during the fermentation period using a Radiometer pH Meter Model 22 (Radiometer; Copenhagen, Denmark).

Sugar analyses

One-ml samples of the yogurt mix or the final yogurt product which had been well mixed prior to sampling were placed in centrifuge tubes containing 1.0 ml of 1.0M acetate buffer pH 4.6 and well mixed. (Reproducibility of the volumetric sampling procedure for yogurt was investigated. The sampling procedure was found to have a variance of 0.0007 and a standard deviation of 0.026.) Precipitated casein was removed by centrifugation at 30,000 × G for 15 min at 4°C in a Servall Superspeed RC-2 Centrifuge. Aliquots of the clear supernatants were analyzed for glucose, galactose and lactose.

Glucose was determined using the Salomon and Johnson reagent as described by Jasewicz and Wasserman (1961) but with the following modifications. To 0.1 ml of sample containing 15–150μg of glucose were added 2.0 ml of water and 1.5 ml of Salomon and Johnson reagent. The color was allowed to develop at room temperature for at least 1 hr and then the absorbancies of the solutions were read at 635 nm using a Zeiss Model PMQ II Spectrophotometer.

Lactose was determined by measurement of the glucose released following hydrolysis with lactase as follows. A 3.0-ml aliquot of Maxilact lactase (Enzyme Development Corp., N.Y.) in 0.1M phosphate buffer at pH 7.0 (5 mg/3 ml) was added to 0.1 ml of sample and incubated for 3 hr at 30°C. The enzyme reaction was terminated by placing the samples in a boiling water bath for 5 min and the solutions then clarified by centrifugation. One tenth ml aliquots were analyzed for glucose by the procedure previously described. Lactose concentrations were calculated by multiplying the glucose concentrations by 1.92.

Galactose measurements were made by an enzymatic method utilizing galactose dehydrogenase. The method used was a modification of a procedure described in the Boehringer-Mannheim catalogue (Boehringer-Mannheim Corp., N.Y.). The assay medium consisted of 0.84 ml 0.1M Tris-HCl buffer pH 8.6; 0.05 ml β-NAD, 5 mg/ml (Sigma Corp.,

St. Louis, Mo.); 0.10 ml sample and 0.01 ml β-galactose dehydrogenase (Sigma Corp.) diluted to approximately 3 U/ml. The assay solutions were incubated at 25°C for 75 min and then read at 340 nm using a Zeiss Model PMQ II Spectrophotometer.

Other analyses

Yogurt samples having a pH of 4.6 were centrifuged at 30,000 x G for 15 min in a Servall Superspeed RC-2 Centrifuge at 4°C to sediment the insolubilized casein. The supernatants were used for lactic acid, acetaldehyde and diacetyl determinations. Lactic acid determinations were made by the procedure of Lawrence (1970). Acetaldehyde was determined by the method of Lindsay and Day (1965) and diacetyl was determined by the method of Pack et al. (1964).

All experiments were carried out in duplicate and each experiment was repeated twice.

RESULTS & DISCUSSION

Acid development

Curves illustrating the decrease in pH which took place in LH and control milks during the preparation of yogurts are presented in Figure 1. Less time was required for the pH to decrease to 4.6 in LH milk than in the control. Faster acid development in milk cultures to which lactase has been added has been reported previously. Gilliland et al. (1972) reported that acid production by lactic streptococci in milk was stimulated by the addition of β-galactosidase while Thompson and Gyuricsek (1974) reported that acid development was increased and set time decreased when yogurts were prepared from lactase-hydrolyzed milks. In our experiments faster acid development in yogurts prepared from LH milk was primarily due to an acceleration in the initial rate of acid production when the lactose was prehydrolyzed. This effect diminished, however, as acidity built up in the product, probably as a result of inhibition of the starter culture organisms due to the rapid production of large amounts of acid. Further decreases in pH took place during storage at 4°C. After 24 hr of cold storage, the pH of control and LH yogurts had declined to 4.39 and 4.42, respectively. During an additional 3–4 wk of storage at 4°C, there was a further decrease in pH to about 4.1 in both products. Decline in pH of yogurts during cold storage has been reported by Tramer (1973) who related this effect to the ability of the starter culture organisms to carry out metabolic processes at cold storage temperatures.

Growth of starter culture organisms

Growth curves of a mixed starter culture consisting of *S. thermophilus* and *L. bulgaricus* in control and LH yogurts are presented in Figure 2.

Patterns of growth of the starter culture organisms were similar in both yogurts. *S. thermophilus* predominated in the early stages of the yogurt fermentation due to a rapid rate of growth which was initiated almost immediately after inoculation into the milk medium while *L. bulgaricus* did not begin to multiply rapidly until the latter stages of fermentation after *S. thermophilus* had reached the stationary growth phase. Similar growth patterns were reported by Bautista et al. (1966) in a discussion of the associative growth of yogurt starter culture organisms. Bautista et al. (1966) reported that the lactobacilli stimulate the growth of the streptococci by liberating essential amino acids from the milk proteins. However, the lactobacilli are more acid tolerant than are the streptococci and thus can grow during the latter stages of the fermentation when growth of the streptococci has been inhibited by low pH. Thus our results show that prehydrolysis of the milk lactose had no effect on the symbiotic growth relationships of the starter culture organisms used in this study.

Sugar concentrations

Concentrations of glucose, galactose, and lactose in LH and control milks prior to fermentation and in the finished yogurt products after 24 hr of storage at 4°C are given in Table 1. There was a 30% decrease in the lactose content of control milk during fermentation and a slight accumulation of galactose. The mixed starter culture appeared to be unable to metabolize all the galactose released through intracellular hydrolysis of lactose and the galactose which was not utilized was released into the medium. Gilliland et al. (1972) observed an accumulation of galactose in milk cultures during growth of *S. lactis* C_{10}. They suggested that *S. lactis* C_{10} was unable to

Table 1—Sugar concentrations in control and LH milks prior to fermentation and in the finished yogurt products after 24 hr of storage at 4°C.

	Control		LH	
	Initial	Final	Initial	Final
Glucose (%)	0.0	0.0	2.6	1.6
Galactose (%)	0.0	0.2	2.3	2.1
Lactose (%)	7.1	5.0	2.1	1.5

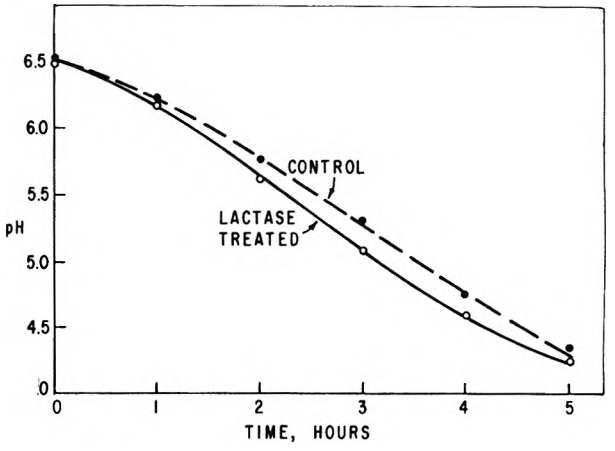

Fig. 1—Changes in pH which occurred during the fermentation of control and lactase-hydrolyzed milks by a mixed yogurt starter culture.

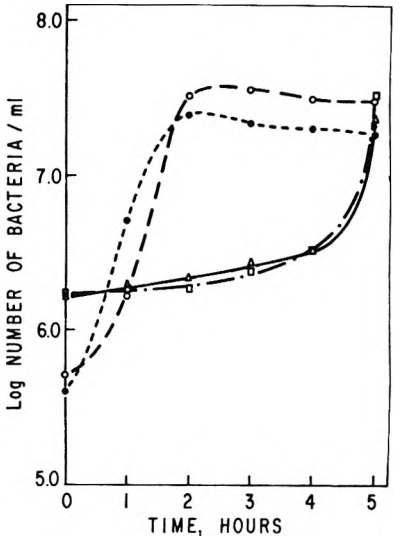

Fig. 2—Growth of a mixed culture of S. thermophilus and L. bulgaricus in control and lactase-hydrolyzed milks. □, L. bulgaricus LH milk; △, L. bulgaricus control milk; ○, S. thermophilus LH milk; ●, S. thermophilus control milk.

utilize all the galactose resulting from catabolism of lactose in milk. Thus it appears that at least certain of the lactic acid bacteria are unable to maximally utilize galactose which is bound in the form of lactose.

Glucose levels in LH milk decreased by 38% during fermentation. In addition, there was a 29% decrease in the lactose content. These results indicate that levels of lactose remaining in lactase-treated milk will be even further decreased during the yogurt manufacturing process. Galactose concentrations, however, showed little or no change during the fermentation process, indicating that free galactose which was made available by prehydrolysis of the lactose was not fermented by the starter culture organisms.

Finished yogurt products prepared from control milk contained about 3.5 times as much residual lactose as did the LH yogurt, i.e., 1.5% in the LH product as compared with 5.0% in the control product. This substantial reduction in residual lactose levels was achieved both by the prehydrolysis treatment and microbial fermentation of some of the remaining lactose. Thus, partial enzymatic hydrolysis of the milk lactose is satisfactory since a 70% hydrolyzed milk yielded a product in which the lactose had been decreased by 80%.

Yogurt flavor compounds

Concentrations of some typical yogurt flavor compounds in pH 4.6 yogurts prepared from control and LH milks are given in Table 3. The LH yogurt contained appreciably more lactic acid than did the control yogurt at pH 4.6. However, acetaldehyde concentrations were about the same while no diacetyl was found in either product. The end products of glucose fermentation by the homolactic lactic acid bacteria have been reported to be lactic, acetic, and formic acids, CO_2, ethanol, glycerol, biacetyl, acetoin, and 2,3-butanediol (Platt and Foster, 1958). Acetaldehyde is also an end product of microbial metabolism in milk cultures (Keenan et al., 1966). Steele et al. (1954) reported that there are marked quantitative but not qualitative differences in the end products produced by *Streptococcus pyogenes, Streptococcus faecalis* and *Lactobacillus casei* when these organisms are grown on galactose as opposed to glucose. They found that when galactose serves as the energy source, a much smaller proportion of the sugar is converted to lactic acid and a proportionately greater amount is converted to acetic acid, formic acid, and ethyl alcohol than when glucose serves as the energy source.

Calculations of the total amounts of glucose and galactose catabolized by the yogurt starter culture organisms in control and LH milks in our experiments are presented in Table 3. Calculations of the amount of galactose catabolized in LH milks are based on the amounts of lactose consumed. Our calculations show that almost twice as much galactose was metabolized in control milk as in LH milk. Thus, our data indicate that the greater quantities of lactic acid produced by the yogurt starter culture organisms in LH milk may have been due to an alteration in the patterns of metabolites produced resulting from the utilization of a greater proportion of the total available sugar in the form of glucose.

In flavor evaluations of the two yogurts by a sensory panel, the LH yogurt was scored significantly higher than was the control product due to the substantially sweeter character of the former resulting from the presence of free glucose and galactose. Although some panel members commented that there appeared to be some other flavor differences between the two yogurts in addition to the difference in sweetness, the substantially greater sweetness of the LH yogurt was the overriding factor in evaluation of this particular product.

Our results have shown that more lactic acid is produced by a mixed yogurt starter culture consisting of *S. thermophilus* and *L. bulgaricus* in yogurts prepared from LH milk. In addition we have observed that a greater proportion of the total available carbohydrate is catabolized in the form of glucose in LH milk. Thus our data indicate that lactase treatment of milk to be used in the manufacture of cultured dairy products may result in changes in the flavor profiles of the resulting products. Such changes may occur as a result of alteration in the types or amounts of metabolic end products produced by the starter culture organisms when the lactose in milk is prehydrolyzed so that a greater proportion of the carbohydrate available for fermentation is metabolized in the form of glucose.

Table 2—Concentrations of some typical yogurt flavor compounds in control and LH yogurts at pH 4.6.

	Lactic acid (%)	Acetaldehyde (ppm)	Diacetyl (ppm)
Control	0.47	2.5	0
LH	0.61	2.4	0

Table 3—Amounts of glucose and galactose catabolized by a mixed yogurt starter culture in control and LH milk during yogurt preparation

	Control			LH		
	Tot. CHO (%)	Glu. (%)	Gal. (%)	Tot. CHO (%)	Glu. (%)	Gal. (%)
Initial	7.10	3.55	3.55	7.00	3.65	3.35
Final	5.20	2.50	2.70	5.20	2.35	2.85
Amount utilized	1.90	1.05	0.85	1.80	1.30	0.50

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This research was supported by a grant from Dairy Research, Inc. Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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CONDITIONING PECANS WITH STEAM TO IMPROVE SHELLING EFFICIENCY AND STORAGE STABILITY

ABSTRACT

Inshell pecans (*Carya illinoensis*, cvs. Stuart and Schley) were conditioned for cracking and shelling by a 3-min atmospheric-steam process and by two processes used commercially. Conditioned and untreated pecans were evaluated for shelling efficiency and for storage stability of the meats. Shelling efficiency was based on percent halves obtained; and storage stability, on changes in peroxide and free fatty acid values, sensory evaluations, and Hunter a/b values during accelerated storage of the halves at 21°C–65% RH. Steam-treated pecans yielded 12–17 percentage points more halves than pecans conditioned by the other processes, and 19 percentage points more halves than the untreated nuts. Halves from steam-treated nuts were less susceptible to oxidative and hydrolytic deterioration during storage.

INTRODUCTION

A MAJOR SOURCE of economic loss in the pecan industry is the low and variable yields of nutmeat halves from commercial shelling operations. These low and variable yields of halves require processing of a large quantity of nutmeat pieces. Since pieces of different sizes are kept separated during grading and packing, a high yield of pieces would require replicates of a number of equipment items in the shelling plant, thereby increasing labor and equipment costs. Because of their small sizes, pieces are difficult to handle; and expensive electronic color sorting equipment is required for grading to remove defective kernels and shell fragments. The variation in yield of halves prevents the development of operating schedules for the most efficient utilization of equipment, labor and facilities.

A low yield of halves also results in losses due to a price differential between halves and pieces. Also, pieces have a shorter shelf life than halves (Woodroof and Heaton, 1962) and are the more susceptible to insect infestation during storage. Depending on market conditions, halves may sell for $0.03–0.15/lb more than pieces. There is a demand for pieces, but it would be more economical to obtain a high, uniform yield of halves, which can be chopped into pieces on a custom-order basis.

In commercial shelling plants inshell pecans are "conditioned" prior to cracking and shelling to improve the yield of halves (Woodroof and Heaton, 1961). The process most widely used involves soaking inshell pecans in large vats of water containing 1000 ppm chlorine for 1–2 hr and then holding them for 12–24 hr before cracking. Another process that is used by a few processors is soaking the pecans in 85°C water for 3–5 min and holding them for 12–24 hr before cracking. These processes sanitize the shells and increase the moisture content of the kernels, making them more pliable and preventing them from breaking into pieces during cracking and subsequent handling. These processes are effective in improving yields of halves, to an extent, but there is still considerable variation and low yields within and among shelling plants. At the present time, typical yields of halves in commercial shelling operations range from 50–80%.

A disadvantage of the conditioning processes currently used is the long process time of 12–24 hr. The chlorine-soak method is also batch-type rather than in-line. Consequently, labor and equipment requirements are excessive, and a considerable amount of plant floor space is required for storing the product in-process. Also, the moisture content of the shelled kernels is high and may be detrimental to quality unless reduced to at least 4.5% before storage (Heaton and Woodroof, 1970). The process could also be a source of product contamination if effective sanitation practices are not strictly adhered to.

Forbus and Smith (1971) found that in conditioning pecans by soaking, kernel moisture content and yield of halves obtained in shelling increased significantly with soak water temperature. Their finding on yields prompted them to recommend that the commercial feasibility for a steam conditioning process be investigated. In the past, some pecan shellers used steam conditioning treatments but discontinued the use because the quality of the nutmeats was adversely affected. Woodroof and Heaton (1961) reported that steam conditioning methods increased the yield of halves but darkened the kernels and gave them a slightly cooked flavor. These adverse effects on quality were caused by subjecting the pecans to steam under pressure and for time periods between 5–15 min.

A steam conditioning process would offer certain economic advantages over existing commercial ones. Total process time could be reduced from 13–24 hr to only several minutes. Steam conditioning would be adaptable to an in-line process and would significantly reduce the labor and equipment costs for processing pecans. In addition to increasing the yield of halves from shelling, a steam process might improve the quality and storage stability of the nutmeats. Heat treatments have been shown to effectively retard rancidity (McGlamery and Hood, 1951).

The purpose of our work was to determine the commercial feasibility of a steam conditioning treatment for pecans. Preliminary work showed that a 3-min atmospheric-steam treatment was the most effective steam process for improving the yield of halves from shelling and for maintaining the quality of the nutmeats. In this paper, we report the effects of selected conditioning treatments, including the 3-min atmospheric-steam process, on pecan shelling efficiency, and quality and storage stability of pecan halves.

MATERIALS & METHODS

IN NOV. 1973, inshell pecans (*Carya illinoensis*, cvs. Stuart and Schley) that had been commercially sized to 15/16 in. diam, cleaned, and separated from the pops were obtained from a commercial shelling plant in Georgia. They were stored, until removed for tests, at 0°C in woven bags that held 100 lb each.

In three replicate experiments, one sample each of the two pecan varieties was conditioned for cracking and shelling by the following treatments: T1–check, or untreated sample; T2–soak 1 hr in 21°C water bath with 1000 ppm chlorine and hold 12 hr before cracking; T3–soak 3 min in 85°C water bath and hold 20 min before cracking; and T4–subject to atmospheric-steam in retort for 3 min and hold 20 min before cracking. Conditioned samples were held at 21°C–65% RH until cracked, and each sample consisted of 100 nuts. T2 and T3 are conditioning processes used in the pecan shelling industry. T4 is the proposed improved process we have developed.

Samples were conditioned, cracked and shelled in the pilot plant under simulated shelling plant conditions. Shelling efficiency, based on percent halves obtained, was determined for each sample. The nutmeats

were dried in a forced-air oven at 49°C until moisture content was about 4%. Physical and chemical parameters indicative of quality were measured before the halves were packed in polyethylene bags for accelerated storage at 21°C–65% RH. Sub-samples were removed from storage at selected times through about 10 months, and quality parameters measured. The parameters were kernel moisture content, color, aroma, peroxide values, free fatty acids, and saturated, unsaturated, and volatile carbonyls.

Processing

Samples were cracked by a commercial automatic pecan cracker from Meyer Machine Co. One sample at a time was dumped into the hopper of the cracker, and a continuous chain with pockets transferred the nuts one at a time to slots in a rotating feed wheel. The feed wheel rotated one nut at a time into position between the plungers of the cracking mechanism and then stopped momentarily. The plungers struck the nut from each end simultaneously, compressing and cracking the shell. As the next nut was rotated into position for cracking, the previous one was discharged into the collection pan on the shelf beneath the discharge chute. The length of stroke of the cracking mechanism was adjustable but held constant for all samples.

After a sample was cracked it was dumped into the hopper of a commercial automatic sheller also from Meyer Machine Co. Cracked nuts were automatically fed into a rotating drum formed by a series of spaced cylindrical rings mounted along the length of the center shaft. As the nuts moved through the drum they were agitated and thrown toward the periphery by metal fingers that protruded perpendicularly from the rotating center shaft. This action loosened the shells from the nutmeats, and both were thrown through the spaces between the cylindrical rings. The kernel-shell mixture dropped through the discharge chute at the bottom of the sheller into a collection pan. The sheller was operated for 5 min after addition of sample.

For each sample the kernel-shell mixture from the sheller was screen-sized by use of a laboratory shaker. The mixture was shaken for 1 min on a screen with mesh diameter of 8/16 in. The shells of the kernel-shell mixture remaining on the screen were removed manually. The halves were selected and dried to about 4% moisture.

Physical analyses

Samples conditioned by each treatment were evaluated for shelling efficiency on the basis of percent halves obtained. The nutmeats in each sample that met the specifications for halves in the United States Standards for Grades of Shelled Pecans (USDA, 1969) were selected by visual examination and counted. Number of halves were converted to percentage of the 200 halves in the original sample.

For each replicate about 120g of halves from each treatment was removed for analysis. 80g of the nutmeats from each treatment was chopped to small sizes, and from this, 8-g portions were placed in 4-oz glass jars. Jars were coded for sample identification, tightly sealed, and left at ambient temperature for 30 min to allow volatile components to equilibrate. Ten subsamples (one per panelist) of each sample were thus prepared and were evaluated subjectively by 10 trained panelists for the presence of oxidized lipid odor. Each panelist examined four subsamples (one per treatment) in a sensory evaluation room with subdued lighting and positive air pressure. They rated odor on a hedonic scale of 1–9 with a score of 1 representing "like very much" and 9 representing "dislike very much."

60 halves per treatment were selected and divided into three subsamples for color difference measurements with a Hunterlab D25D Measuring Unit. Each subsample of 20 halves was placed in the 2-in. diam plexiglas sample holder, and the L, a, and b values determined from four positions; the sample holder was rotated 90° between readings.

A 5-g subsample of nutmeat from each treatment was chopped and sized to ca 0.32 cm. Percent moisture was determined on ca 2g of the sized nutmeats after it was dried to constant weight at 96–99°C and 100 mm Hg (AOAC, 1970).

Chemical analyses

Nutmeats that had been examined psychometrically were composited according to treatment. Each composite was macerated with an aluminum block and plunger and a Carver Laboratory Press. About 70 kg/cm^2 pressure was applied to force the nutmeats through the holes (0.08 cm diam) in the block. The macerate was collected in glass beakers, transferred to centrifuge tubes, and centrifuged at 10,000 × G for 10 min. The separated oils were then decanted into glass vials, flushed with nitrogen, and stored at 4°C until analyzed. Duplicate analyses for peroxides, free fatty acids, saturated carbonyls, unsaturated carbonyls and volatile carbonyls were completed within 24 hr after the nutmeats had been removed from storage.

Peroxide values, expressed as milliequivalent of oxygen per kilogram of oil (meq O_2/kg oil), were determined according to Holloway (1966). Values were determined from a standard curve of optical density (x) vs μg of Fe (y), and from the corresponding regression equation $y = 36.426x + 0.2795$ ($r = 0.998$).

Free fatty acid values were determined according to Triebold and Aurand (1963). 10g of oil was titrated to neutrality with 0.1N KOH. Results of such titrations are expressed as mg of KOH required to neutralize the free fatty acids present in 1g of oil.

The formation of saturated and unsaturated carbonyls in the oils was determined according to Berry and McKerrigan (1958); the absorbance of the 2,4-dinitrophenylhydrazones formed was measured spectrophotometrically. Analysis for volatile carbonyl production was based on the above procedure with the following modification: Smaller size apparatus was used to hold a sample of ca 5g instead of 100g; nitrogen flow was 60 ml/min; and the entire condensate was dissolved in 5 ml of methanol for color development and analyzed spectrophotometrically. For highly oxidized pecan oil, the modified procedure gave a volatile carbonyl index of 3873, which agrees well with the index of 2320 for oxidized soya-bean oil (Berry and McKerrigan, 1958).

Statistical analyses

Differences in shelling efficiency due to conditioning treatment and pecan variety were determined by analysis of variance and Duncan's New Multiple Range Test (Steel and Torrie, 1960).

The relationships between the quality parameters and storage times were difficult to determine because the nutmeats did not deteriorate as rapidly as expected. In order to conserve samples, we analyzed a smaller number of replicates during the later stages of the study; also, some of the parameters were measured at storage times that differed for the two varieties. After 24 wk storage only one replicate was analyzed for peroxide and free fatty acid values, saturated carbonyls, unsaturated carbonyls, volatile carbonyls, and hedonic ratings. All of these parameters except the hedonic ratings, were run through 42 wk for Stuarts and 44 wk for Schleys. Hedonic ratings and color measurements were obtained through 36 wk for the Stuarts and 39 wk for the Schleys.

From the entire data set, all possible correlations between variables were calculated for each variety and for the varieties combined. Also, for each variety, the regressions of each parameter as a function of storage time were analyzed. For each variety and treatment, means of the parameters were plotted against storage time.

From the entire data set, we selected four of the parameters for detailed discussion on the effects of the treatments and accelerated storage on overall nutmeat quality. The parameters were peroxide values, free fatty acid values, hedonic ratings, and Hunter a/b ratios. Results of the carbonyl analyses are not discussed because they closely paralleled those of the peroxides and free fatty acids. Selection was based on the correlation and regression analyses and examination of the plots of these parameters vs. storage time.

Because of the unequal number of replicates for the measurement of some parameters, and because some of the parameters were measured at different storage times for the two varieties, we analyzed the experimentally determined means rather than the values calculated from the regressions. Differences in means at the 5% probability were evaluated by lsd tests (Steel and Torrie, 1960).

RESULTS & DISCUSSION

OUR PRIMARY CRITERIA for evaluating the commercial feasibility of the atmospheric-steam conditioning process for pecans were the effects of the treatments on shelling efficiency and on product quality and storage stability. Shelling efficiency was based on percent halves recovered under conditions simulating commercial shelling operations. Evaluation of treatment effects on the quality and storage stability of the product was based on peroxide and free fatty acid values, hedonic ratings of aroma, and color. Samples were stored at 21°C–65% RH, conditions that accelerate the deterioration of quality.

Shelling efficiency

In the two-way analysis of variance for shelling efficiency, the F value of 2.98 for varieties was not significant at the 5% level; therefore the means for the treatments (Table 1) were

computed over both varieties. In the analysis of variance, the F value of 22.05 for treatments was significant at the 5% level. Samples conditioned by the 3-min live-steam treatment (T4), yielded an average of 91.42% halves, which was significantly greater than the means for all other treatments (Table 1). The difference between treatment means for T2 and T3 (commercial treatments) was not significant at the 5% level. The treatment mean for T1 was not significantly different from that for T2, but was significantly less than that for T3.

The percent yield of halves for T1 (72.67) was greater than that expected for pecans not conditioned prior to cracking. The increased yield may have been due to storage of the pecans at 0°C, which maintained the kernel moisture content at about 4%. This moisture content was higher than that of nuts stored at ambient temperatures as in commercial practice.

Table 1—Effect of conditioning treatment of pecans on shelling efficiency based on percent halves[a] obtained

Conditioning treatment	Rep	Percent halves: Variety Stuart	Variety Schley	Treatment means[b,c]
T1—check, untreated	1	75.50	77.50	
sample	2	68.50	71.00	72.67d
	3	74.50	69.00	
T2—soak 1 hr 21° C water	1	70.00	76.50	
1000 ppm chlorine, hold	2	69.50	72.50	74.83de
12 hr 21° C—65% RH	3	83.00	77.50	
T3—soak 3 min 85° C water,	1	72.50	83.50	
hold 20 min 21° C—65% RH	2	73.50	86.00	79.25e
	3	80.50	79.50	
T4—live steam 3 min,	1	86.00	95.50	
hold 20 min 21° C—65% RH	2	91.50	89.00	91.42f
	3	91.00	95.50	

[a] Number of halves counted for each sample converted to percent of 200 halves in the 100-pecan sample.
[b] Percent halves averaged over both varieties since difference between varieties not significant.
[c] Mean separation in column by Duncan's multiple range test, 5% level.

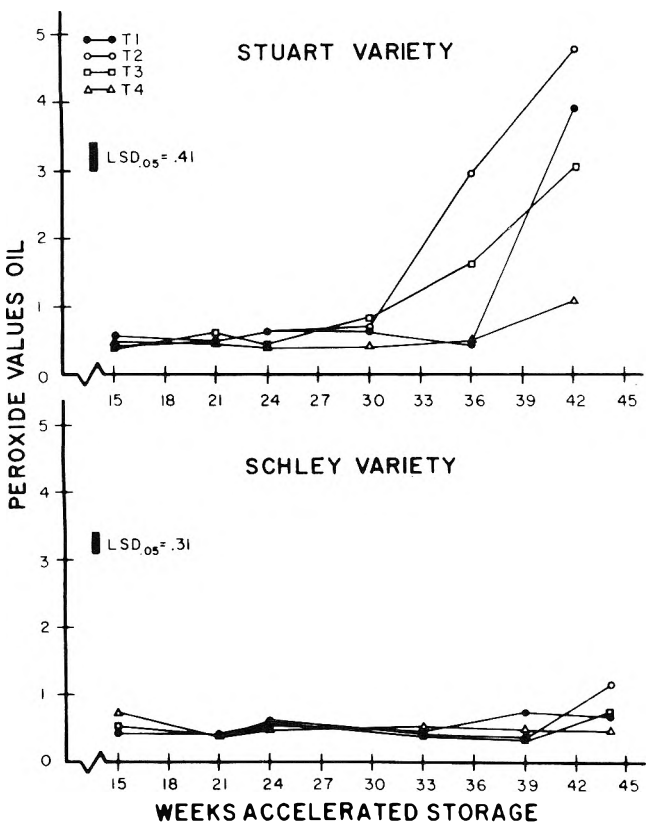

Fig. 1—Effect of accelerated storage at 21° C—65% RH on peroxide values of Stuart and Schley pecans conditioned by four treatments. Peroxide values of oil are expressed as meq O_2/kg oil.

Quality and storage stability

Deterioration in the quality of pecan nutmeats during storage is attributable to lipid oxidation and hydrolysis. Increases in peroxide and free fatty acid values are indicative of the former and latter reactions, respectively. Increases in the hedonic ratings of aroma reflected a decline in quality. Darkening effect due to processing and storage was indicated by increases in the Hunter a/b ratio, the ratio of red to yellow, and indicates a decline in quality.

Peroxide values. Differences in peroxide values due to treatments were not significant through accelerated storage of 30 wk for the Stuart variety and 33 wk for the Schleys (Fig. 1).

Beyond 30 wk storage of Stuart pecans, peroxide values were significantly higher for T2 than for all other treatments. At 36 wk storage of Stuart pecans, the peroxide value for T3 was significantly higher than that for T1 or T4; but at 42 wk the value for T3 was significantly less than that for T1. At 36 wk storage, the peroxide values for T1 and T4 were not significantly different; but at 42 wk, the peroxide value for T4 was significantly lower than that for any other treatment.

At 39 wk storage of Schley pecans, peroxide values for T2, T3 and T4 were not significantly different. The peroxide value for T1 was not significantly different from that for T4, but was significantly greater than the peroxide values for T2 and T3. At 44 wk storage, the peroxide value for T2 was significantly greater than that for any other treatment. Differences in peroxide values between T1, T3, and T4 were not significant.

These data show that peroxide values for the Schley variety did not increase as fast, nor to as high values as those for the Stuarts. If the storage time for Schleys had been extended, we assume that their values would have increased to values like those reached by the Stuarts in this study. The data also suggest that Schley pecans are less susceptible to oxidative deterioration than Stuart pecans.

Free fatty acid values. During 24 wk storage, no significant differences due to treatment were apparent in free fatty acid values for either the Stuart or Schley varieties (Fig. 2).

At both 30 and 36 wk storage of Stuart pecans, differences in free fatty acid values for T2, T3 and T4 were not significant at the 5% level. At 42 wk storage, free fatty acid values for T2 and T3 were not significantly different, but they were significantly higher than the value for T4. Free fatty acid values were significantly less for T1 than for all other treatments at 30, 36, and 42 wk storage.

At 33 wk storage of Schley pecans, free fatty acid values for T2, T3, and T4 were not significantly different, but they were significantly less than the value for T1. At 39 and 44 wk storage, free fatty acid values were significantly higher for T3 than for all other treatments. At 39 wk, the free fatty acid value for T1 was significantly greater than that for either T2 or T4, and the difference between T2 and T4 values was not significant. At 44 wk, the free fatty acid value was significantly less for T4 than for all treatments except T1.

Figure 2 shows that fatty acid production did not increase at as fast rates nor to as high values in Schleys as in Stuarts. This suggests that Schley pecans are less susceptible to hydrolytic deterioration during storage than Stuarts.

Hedonic rating. Differences in the mean hedonic ratings due to treatments were not significant for either variety through 24 wk storage (Fig. 3).

Beyond 24 wk storage of Stuart pecans, hedonic ratings for T2 increased (indicating a decline in quality) at a faster rate than those for other treatments. The mean hedonic rating for T2 was significantly different from that of any other treatment at 30 and 36 wk storage. Mean hedonic ratings for T1, T3, and T4 were not significantly different at 30 wk storage. The mean hedonic ratings for T1 and T3 were not significantly different at 36 wk storage. The mean hedonic rating for T4 was significantly different from that of any other treatment at 36 wk storage.

At 33 wk storage of Schley pecans, the mean hedonic ratings for T1, T2, and T3 were not significantly different. Although the mean hedonic rating for T4 was significantly different from that for T1, it was not significantly different from that for either T2 or T3. At 39 wk storage, mean hedonic ratings for T2, T3 and T4 were not significantly different, but they were significantly different from the value for T1. Between 33 and 39 wk, the mean hedonic rating for T1 increased at a faster rate than that for any other treatment.

Comparison of mean hedonic ratings and mean peroxide values were made according to variety, treatment, and storage period. Differences in peroxide values due to treatments were not significant until 30 wk storage for the Stuarts and 39 wk storage for the Schleys. However, the sensory evaluation panel detected differences in the aroma of the samples stored only 30 wk for the stuarts and 33 wk for the Schleys. This indicates that the panel was efficient in detecting off-odors due to deterioration in quality.

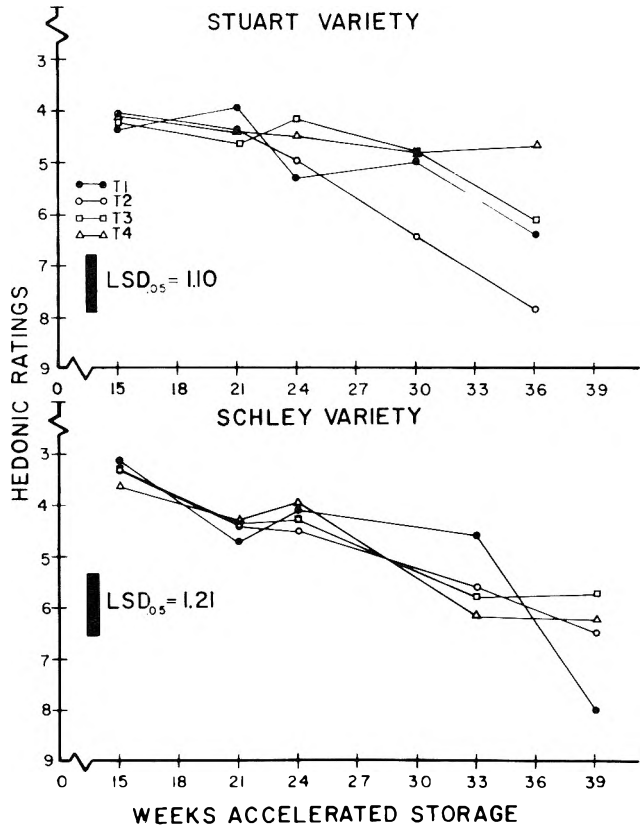

Fig. 3–Effect of accelerated storage at 21°C–65% RH on hedonic ratings of Stuart and Schley pecans conditioned by four treatments.

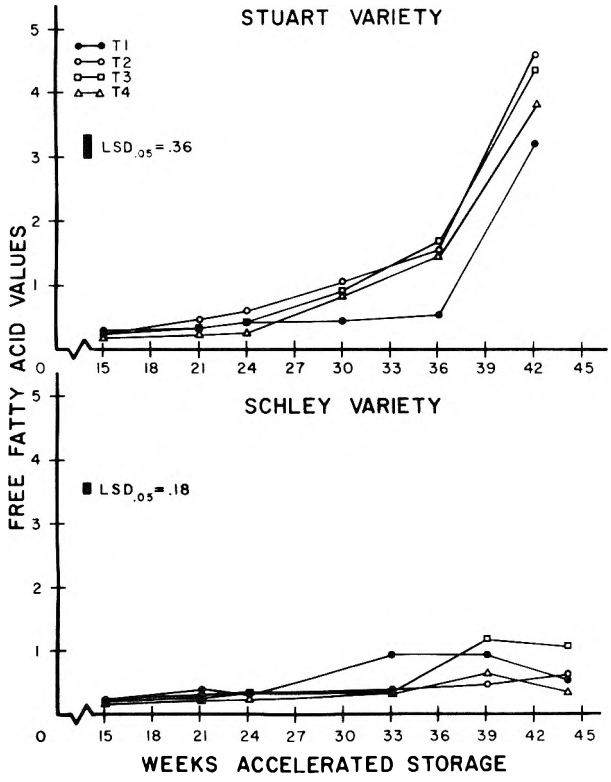

Fig. 2–Effect of accelerated storage at 21°C–65% RH on free fatty acid values, which are expressed as mg KOH required to neutralize the free fatty acids present in 1g of oil.

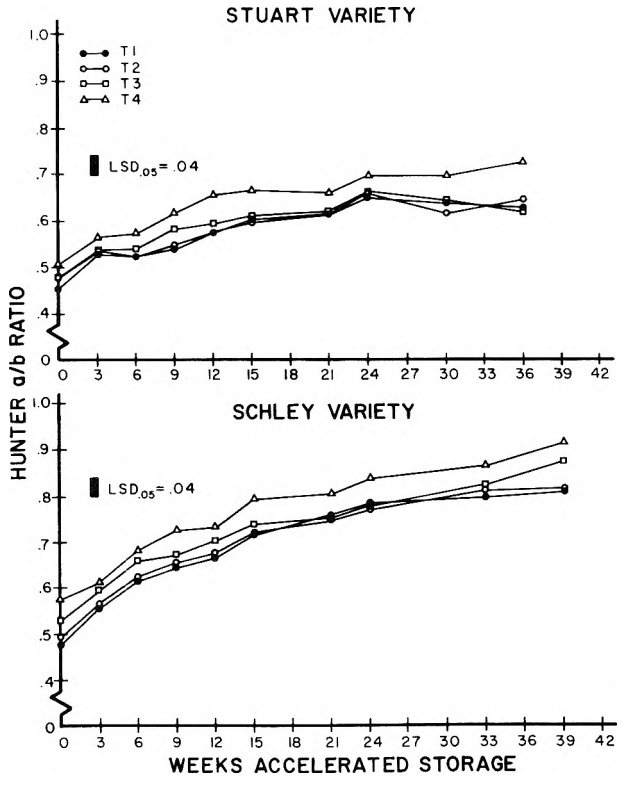

Fig. 4–Effect of accelerated storage at 21°C–65% RH on the Hunter Color a/b ratio of Stuart and Schley pecans conditioned by four treatments.

Color. The a/b ratios for all treatments and for both varieties followed essentially the same trend during storage (Fig. 4).

For practically all storage periods, the a/b ratios were significantly higher for T4 than for the other treatments. However, the a/b ratios did not increase at a significantly faster rate for T4 than for the other treatments. These results indicate that color of the pecans was affected more by T4 than by the other treatments, but that storage stability of the pecans as measured by reflectance was about the same for all treatments.

The a/b ratios for the Schley samples were higher initially and increased at a more rapid rate to higher final values than those for the Stuart samples. This indicates that Schley pecans are more susceptible to color changes during storage than Stuart pecans. Also, the a/b ratios for samples conditioned by heat (T3 and T4) were higher initially than those for samples from the other treatments, especially in the Schley variety. The higher ratios for T3 and T4 can probably be attributed to the thinner shells of the Schley variety, which allow greater heat penetration.

Pecans conditioned by T4 underwent a slight color change during processing. However, the change would not have reduced the value of the pecans and was difficult to detect with the naked eye. Pecans conditioned by T4 appeared more uniform in color than those conditioned by the other treatments.

CONCLUSIONS & RECOMMENDATIONS

WE FOUND on the basis of shelling efficiency and storage stability, that the 3-min atmospheric-steam conditioning process for pecans was superior to conditioning processes presently used in industry. Pecans conditioned by the 3-min live-steam treatment yielded an average of from 12–17 percentage points more halves than pecans conditioned by the commercial methods, and 19 percentage points more halves than untreated nuts.

Immediately after processing, the quality of nutmeats was about the same for all conditioning treatments. Also, nutmeats steam-treated in the shell were generally not as susceptible to oxidative and hydrolytic deterioration during storage as those from nutmeats conditioned by the commercial-type treatments. Although the meats of steam treated pecans were slightly darker than those of nuts conditioned by other treatments, they were more uniform in color. The color change would not have caused the nuts to be downgraded in quality or selling price.

Commercial shelling plant operators could expect results similar to those obtained in this study since the work was conducted under simulated commercial conditions. Before the steam process is adopted by industry, it must be adapted for and tested in a commercial shelling plant, and the findings of our study verified. Adoption of the process by the industry could significantly reduce the marketing cost of pecans.

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Ms received 10/10/75; revised 12/19/75; accepted 12/25/75.

We thank Ruel L. Wilson Jr. for assistance with the statistical analyses, Brenda G. Lyon for conducting the sensory evaluations, and Judy E. Smith for laboratory assistance.

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OAT PROTEIN CONCENTRATES FOR BEVERAGE FORTIFICATION

ABSTRACT

Up to 4% oat protein concentrates can fortify either neutral or acidic beverages. The concentrate was produced in good yield from both defatted and nondefatted oat groats by alkaline extraction. If desired, the gum and water-soluble protein fractions can be removed by a preliminary water extraction yielding a concentrate with a higher percentage of protein. Nitrogen solubility of protein concentrate from defatted groats in dilute phosphoric acid was considerably higher than one from nondefatted groats. Fortified acidic beverages (pH 3) prepared with oat concentrate from laboratory ground groats were slightly astringent in taste. However, astringency was eliminated if the concentrate was produced from commercial oat flour (heat treated during production). Milklike and breakfast-type beverages fortified with oat protein concentrate would be nutritious, palatable and easily prepared and flavored.

INTRODUCTION

NUTRITIONAL QUALITY of consumer foods and beverages can be improved by adding vitamins, protein and other supplements to them. To be well accepted, these additives must not adversely affect native taste and original qualities. Fortifying beverages with protein from various sources is not a new concept. Holsinger et al. (1973) described the fortification of soft drinks with 0.5–1% undenatured cheese whey protein. Also, such protein combined with orange juice makes a beverage somewhat similar in appearance and flavor to orange juice but with a protein content about equal to that of milk and offers the benefits of both (Anonymous, 1973). A lipid-protein concentrate derived from soybeans has been incorporated into beverages with excellent qualities (Mustakas, 1974).

Because of their ubiquitous distribution in temperate regions of the world, oats could be an excellent source of a quality protein suitable for fortifying beverages and food products. One leading U.S. oat company has announced a multi-use oat powder from whole oat groats with a protein content of 15% (Anonymous, 1974). Oat protein concentrates produced by alkali extraction of ground groats (Cluskey et al., 1973) have a bland taste and a high solubility around pH 2.5 (Wu et al., 1973). Development of an oat protein concentrate possessing a protein content of approximately 75% and its experimental use in palatable nutritious beverages is reported here. Also, an acid-soluble fraction from the oat concentrate was tested for fortifying many types of popular beverages, including ades and carbonated drinks.

MATERIALS & METHODS

Oats

Oat flour was a gift from the National Oats Company, Cedar Rapids, Iowa. This commercial blend flour had been heat processed at the mill and had a protein content (nitrogen × 6.25) of 17.9% (dry basis). Garland oats (Lot BH 474) purchased from Interstate Seed and Grain Co., Fargo, N.D., had been grown in Minnesota in 1970 and had a protein content of 17.2% (dry basis). Dal oats, a gift from H.L. Shands, Dept. of Agronomy, University of Wisconsin, Madison, grown in Wisconsin in 1971, had a protein content of 17.8% (dry basis) and represented a high-protein variety with a high oil content.

After the Garland and Dal oats were dehulled in an Alpine pin mill at 1445 rpm, groats were separated from hulls by screening and aspiration. Groats were then ground in a hammer mill; some were defatted with 1-butanol, followed by a hexane wash to remove any remaining solvent, whereas some were defatted only with hexane.

Protein concentrate

The procedure for making protein concentrate and by-products from groats is diagrammed in Figure 1. Ground oat groats, 25–300g, and water in a 1:6 ratio were mixed well before the slurry was stirred for 25 min and then centrifuged for 10 min at 3300 × G in a Sorvall laboratory centrifuge. The water extract containing water-soluble protein and gum was decanted and freeze dried. Water was then added to the residue to restore the original volume of the slurry, 10M sodium hydroxide was added to pH 9 and the slurry was stirred and centrifuged again. Protein concentrate A (first sodium hydroxide extract, Fig. 1) was recovered by neutralizing the alkaline supernatant with 6N hydrochloric acid and freeze drying the neutralized dispersion. The procedure can be terminated at this point with neutralization and drying of the residue. Pilot plant runs, using 12 kg of oat groats, were also made.

If additional fractions are desired, processing continues. The original volume of the slurry is again restored by adding water to the alkaline residue and by increasing pH to 9 with 10M sodium hydroxide. This alkaline slurry is then stirred, passed through 100-mesh bolting cloth and centrifuged; the supernatant is neutralized to get a second sodium hydroxide extract. Finally, the starch fraction is neutralized and dried. The concentrate resulting from the second sodium hydroxide treatment has about one-half the protein contained in the protein concentrate A. The pH optimum for protein extraction from commercial groat flour

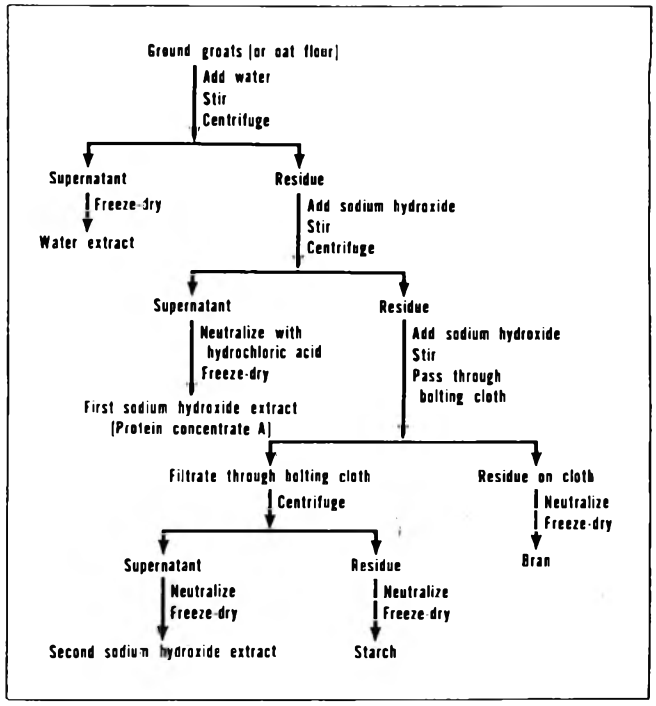

Fig. 1—Schematic diagram for making oat protein concentrates and by-products from ground groats or oat flour by alkaline extraction.

was 10 rather than 9, the optimum for groats ground in the laboratory.

An alternate, shorter method to produce oat protein concentrate is given in Figure 2. The oat flour is slurried with water and brought to pH 10 with 10M sodium hydroxide. After stirring for 25 min, the slurry is centrifuged and supernatant pH lowered to 5.5 with 6N hydrochloric acid. The residue cake is neutralized to pH 7 with acid and dried. This residue contains starch, bran and residual protein. The protein-containing supernatant is next centrifuged (16,000 × G), and the precipitated concentrate collected and freeze dried. When the protein concentrate is prepared in this manner, the term precipitated protein concentrate B is used. The supernatant containing the gum and the water-soluble proteins is freeze dried. If the alkaline supernatant is adjusted to neutrality and dried, the product is referred to as protein concentrate C.

Analytical methods

Protein content was calculated from duplicate micro-Kjeldahl analyses by multiplying percent nitrogen by 6.25 and expressed on a dry basis. Starch was measured by a polarimetric procedure (Garcia and Wolf 1972). Total neutral carbohydrates of acid-hydrolyzed samples were determined by a GLC method (Sloneker, 1971). Ash contents were determined by AACC *Approved Methods* (1971).

Each sample for amino acid analysis was hydrolyzed in refluxing constant-boiling hydrochloric acid for 24 hr, evaporated to dryness and dissolved in pH 2.2 citrate buffer. Amino acids were separated in a Beckman Spinco Model 121 amino acid analyzer, and data were computed automatically according to a procedure by Cavins and Friedman (1968).

Protein efficiency ratio (PER) assays were conducted by the WARF Institute, Inc., Madison, Wis. Dal groats and Dal protein concentrate were each fed to 10 weanling rats for 4 wk. Standard casein served as the control. PER values were corrected to the casein value set at 2.5.

Table 1—Yield and composition of Garland (G) and commercial blend (F) oat protein concentrates and by-products (% dry basis)

	Yield		Protein (N X 6.25)		Ash	Starch	Total neutral carbohydrate of hydrolyzate
Fraction	G	F	G	F	G	G	G
Groats			23.3	17.6	2.2	50.4	
Water extract	7	5	25.6	15.4	7.9	4.7	55.8
Protein conc A	22	16	76.7	74.2	5.2	0	4.9
Second extract	4	4	37.6	39.6	14.1	0	31.9
Bolting cloth residue	9	17	14.1	12.5	8.8	3.1	49.6
Layer above starch	8	51	2.3	1.6	2.6	79.6	91.9
Starch fraction	43		0.2		0.7	97.4	103.5

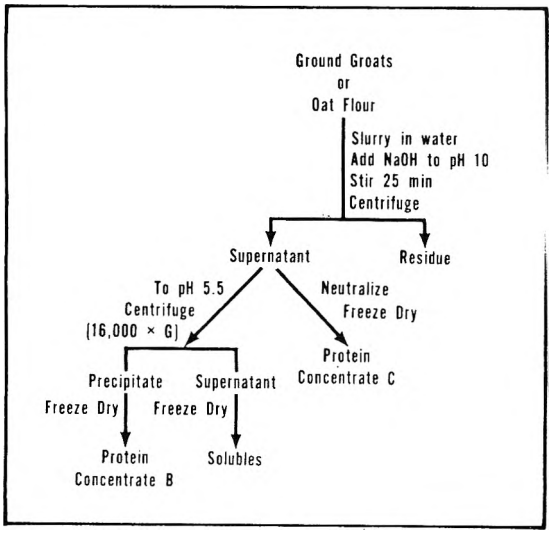

Fig. 2—Schematic diagram illustrating preparation of protein concentrates from ground groats or oat flour.

Solubility

The solubility of protein concentrate at various pH values was determined by mixing 0.1 (or 0.4) g of concentrate with 10 ml of acid or alkali solution. The mixture was stirred 25 min magnetically before being centrifuged at 3300 × G for 10 min. The supernatant was analyzed for nitrogen by a micro-Kjeldahl procedure.

Preparation of beverages

Essentially two types of beverages were prepared: milklike (a neutral type) and breakfast drink or ade (acidic types).

The milklike beverage was made with oat protein concentrate from the commercial oat flour that contained the gum fraction, in total or in part. Oat concentrate was added to water in the ratio of 2.5g to 100 ml water. Since the protein concentrate was not 100% protein, the protein content in the drink was based on the protein value calculated from Kjeldahl nitrogen determinations. Sucrose was added and if desired a flavoring. The ingredients were then mixed in a Waring Blendor for 1–2 min. Dispersion viscosity was varied by increasing or decreasing the amount of the protein concentrate.

Protein concentrate was also used with commercial powdered drink bases, such as chocolate- or strawberry-flavored milk shakes. Rather than adding milk to the base, the concentrate and water gave an acceptable and palatable drink.

Xanthan gum, a natural biopolysaccharide, was added in some beverage preparations to stabilize the suspension of concentrate particles. Xanthan gum is a food-grade product manufactured by Kelco Co., Clark, N.J.

To regulate viscosity of the milklike drinks, synthetic mixtures of concentrate and gum-soluble protein fraction were investigated. The ratio of 1:8, gum fraction:concentrate, was considered optimum for beverages simulating a consistency of whole milk.

Beverages were also prepared from acid extracts of the concentrate or from the concentrate solids in a pH 3 medium. Acid extracts were prepared by mixing 4g of solid concentrate with 100 ml of dilute phosphoric acid. The pH was adjusted to 3 by adding 1M phosphoric acid. The mixture was stirred magnetically for 25 min and then centrifuged at 3300 × G for 10 min. The extract was sweetened, and if desired, flavored and carbonated. Because of the unpleasant mouth feel factor in some acid extract beverages, those that incorporated protein concentrate C were favored. The breakfast or ade-type beverage, prepared with protein concentrate C, was generally adjusted to pH 3 with 1M phosphoric acid. Acidic-type beverages were also prepared with malic acid. Malic acid crystals, concentrate, sucrose and water were blended to yield a mildly flavored drink. Other protein-fortified beverages were prepared by mixing the desired level of solid concentrate with uncarbonated soft drink syrup, blending and carbonating in a seltzer bottle.

Astringency

An astringent aftertaste was common in acidic beverages fortified with oat protein concentrate prepared from laboratory-milled groats. However this aftertaste was not present in concentrates prepared from commercially available groat flour. During processing, commercial groat flour is given a heat treatment that evidently removes the astringency factor. For this reason, concentrates prepared from commercial groats were used almost exclusively in acidic-type beverages.

Organoleptic evaluation

The protein-fortified beverages were scored by 10 experienced tasters at the Northern Laboratory. Suitable controls were used. Flavor scoring was from 1 to 10 with 10 being the most bland. An intensity value of 1.0 as weak, 2.0 as moderate and 3.0 as strong was given to the flavor description by each taster. Any flavor can be added to the bland product.

Yield and composition

The yield and composition of Garland protein concentrate and of by-products are shown in Table 1. Water extraction removed oat gum, as indicated by the high carbohydrate content, which causes extracts to

be difficult to screen because of their high viscosity. The water-soluble oat albumins are extracted by water also. Protein concentrate A has 76.7% protein, 5.2% ash, no starch and 4.9% carbohydrate. The second sodium hydroxide extraction gave another fraction high in protein and significantly reduced protein contents of the subsequent bolting cloth residue and starch fractions. When an alternative procedure is used–i.e., when the supernatant from centrifuging the first sodium hydroxide slurry was adjusted to pH 5.5 to precipitate the protein–this precipitated concentrate had a higher protein content, 89.9%, but a lower yield, 19%.

Fractionation of a commercial groat flour of 17.6% protein gives a lower yield than Garland groats of protein concentrate A, 16%, a higher bolting cloth residue, 17%, and comparable water extract and second sodium hydroxide extracts. The percentage protein in the first and second sodium hydroxide extracts and in the bolting cloth residue is nearly the same as that from a high-protein groat Garland. However, the protein content of the water extract was 10% lower than in the high-protein groat water-soluble fraction.

Amino acid composition

The amino acid compositions of defatted Garland groats, the water extract and the protein concentrate of the defatted groats and FAO pattern (FAO/WHO, 1965) are given in Table 2. The data for the same lot of defatted Garland groats were reported previously (Wu et al., 1973) and are included here for comparison. The water extract has high lysine and half-cystine contents. Defatted groats and the protein concentrate have similar amino acid composition and both have good levels of lysine, total sulfur amino acids and other essential amino acids compared with the FAO pattern. Removal of the water extract from the defatted Garland groats apparently caused little loss in essential amino acids for the protein concentrate because the water extract only accounted for a small part of the oat proteins (Table 1). The amino acid profile of the concentrate from commercial groats was essentially the same as that reported here.

PER values, corrected to standard casein set at 2.50, amounted to 1.87 for nondefatted Dal groats and 1.84 and 1.82 for two protein freeze-dried and spray-dried Dal groat protein concentrates respectively. Evidently, any processing involved in preparing protein concentrates does not decrease their nutritional value.

Solubility

Nitrogen solubility of Garland protein concentrate in 0.01M citric acid and in 0.01M phosphoric acid is plotted in Figure 3. Either hydrochloric acid or sodium hydroxide solution was used to adjust pH to the desired value. The minimum solubility of the protein concentrate in both citric and phosphoric acids is near pH 5.5. Solubility increases considerably in phosphoric acid and in citric acid at pH 2. Good solubility was also observed above pH 8. In general, phosphoric acid dissolved more Garland protein concentrate at all pH values than citric acid, except near the minimum solubility regions. Therefore, phosphoric acid is a better medium than citric acid for acidic-type beverage fortification based on solubility of a protein concentrate. Similarly, a solubility index curve was constructed for hexane-defatted Dal groat concentrate; the percent nitrogen solubilized at pH 2 was similar to that when butanol-defatted Garland concentrate was used.

Nitrogen solubility data were also obtained for protein concentrates from nondefatted commercial groat flour. Two concentrates were prepared as outlined in Figure 2; one contained the gum-solubles fraction, i.e., the total isolated sodium hydroxide extract C; the other, precipitated protein concentrate B did not contain this fraction (Fig. 3). Phosphoric acid 0.01M was the solvent; the starting solids concentration was 4%. Concentrate C containing the gum fraction was much less soluble in the pH 2–3 range than the others. Also, the minimum solubility range of concentrate B was broader (pH 4–6), and slightly higher in percent nitrogen solubility. The commercial protein concentrate C showed solubility characteristics similar to those of the Garland concentrate in 0.01M phosphoric acid between pH 4–6.

Solubility of Garland protein concentrates from defatted and nondefatted groats in phosphoric acid between pH 2 and 3 is a function of initial solid concentration (Table 3). Nitrogen solubility of concentrate decreases as the starting solids concentration increases around pH 2. The Garland concentrate from defatted groats had better solubility than that from nondefatted groats; the difference in solubility is especially large at 3% or higher initial solid concentration.

Heat stability

When a 2% water-soluble fraction from Garland groats at pH 6.4 was boiled in water for 10 min, the respective percentages of nitrogen percipitated were 45 and 32 for nondefatted and defatted samples. However when the Garland protein concentrates were boiled in 0.1M citric acid, pH 2.1, no precipitation occurred. Similar results were obtained with 0.1M phosphoric acid.

Table 2–Amino acid composition of groat protein fractions (g/16g nitrogen) and comparison with FAO standard pattern

	Defatted Garland groats			
Amino acid[a]	Groats	Water extract	Protein conc A	FAO pattern
Lysine	4.1	6.5	3.8	4.2
Histidine	2.2	2.0	2.5	
Ammonia	2.6	2.6	3.0	
Arginine	7.0	4.6	8.1	
Aspartic	8.1	13.0	8.5	
Threonine	3.3	4.3	3.5	2.8
Serine	4.6	7.7	4.8	
Glutamic	20.9	13.8	25.7	
Proline	6.1	5.4	4.8	
Glycine	4.8	9.4	4.6	
Alanine	4.4	5.7	4.7	
Half-cystine	1.9	6.8	2.0	2.0
Valine	5.2	4.1	6.2	4.2
Methionine	1.7	1.5	1.9	2.2
Isoleucine	3.8	2.6	4.7	4.2
Leucine	7.4	5.3	8.5	4.8
Tyrosine	3.9	5.3	4.4	2.8
Phenylalanine	5.3	2.9	6.5	2.8

[a] Amino acid content of commercial groats is similar to that of Garland.

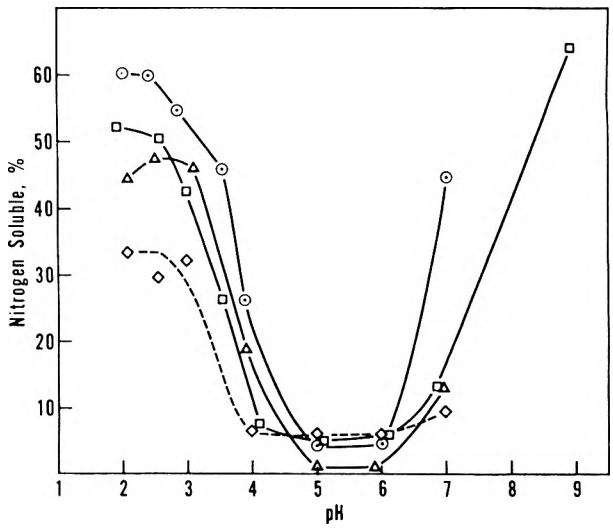

Fig. 3–Nitrogen solubility of Garland groat protein concentrate A in 0.014M citric acid □ and in 0.010M phosphoric acid ○ and of commercial protein concentrates in 0.01M phosphoric acid. pH adjustment was made with hydrochloric acid or sodium hydroxide solution. The Garland protein conc A has 77% protein; 1% solid was used at the start. Commercial protein conc B △ has 77% protein; commercial conc C ◇ has 56%; 4% was the starting solids concentration. Conc C was prepared at pH 9 rather than pH 10 as were the others.

RESULTS

Beverage evaluation

Protein-fortified beverages were submitted to experienced taste panelists. Two different drinks were taste tested; one contained a concentrate from laboratory-milled high-protein groats; the other was prepared with a concentrate from commercially available groat flour. The former were usually acidic-type drinks, whereas the latter were both acidic and neutral in pH. Both citrate and phosphate media were used in the preparations. Because the phosphate-based fortified beverages were judged more bland than the citrate-based one, phosphoric acid was used more frequently. Further, beverages that incorporated concentrates from nondefatted groats were more bland than those from defatted groats.

Beverages prepared from centrifuged acid extracts of solid concentrates were evaluated by a taste panel (Table 4). Flavoring was added to some; sucrose was the sweetener for all. A decrease in flavor score, blandness from 7.3 to 4.2 scale units was observed when the protein level increased from 0 to 2.7% in the beverage that contained Dal concentrate A from hexane-defatted groats in 0.01M phosphate, pH 3.0. A smaller change in blandness was observed when nondefatted Dal concentrate in 0.04M phosphate at pH 3 was used. No undesirable flavor was detected in the protein-fortified beverages; the lower score was due primarily to astringency. Beverages fortified with protein from ethanol-, 1-butanol- and hexane-defatted Dal groats all had the same astringency level; however, the beverage derived from ethanol-defatted groats had the best flavor score. For comparison, sodium caseinate in 0.01M phosphate elicited a similar taste response as that from the oat protein concentrate from ethanol-defatted groats; however, the astringency of casein was greater than that of the oat protein.

The effects of phosphate concentration and carbonation on the organoleptic properties of protein-fortified beverages with 13% sugar at pH 3.0 are also shown in Table 4. There was no change in solubility of Dal protein concentrate from nondefatted groats, nor flavor score and astringency intensity, when the phosphate concentration was increased from 0.04 to 0.06M. However, when phosphate concentration was increased to 0.1M, the beverage had a better flavor score (more bland), less astringency and lower protein concentration. Carbonation had no effect on protein solubility, flavor score and astringency intensity value in a beverage from Dal concentrate in 0.06M phosphate. The difference in flavor score was only 1 unit between the carbonated, flavored control and the protein-fortified beverage. The source of the concentrate, Dal and Garland oats, does not affect flavor score or astringency intensity when the same phosphate concentration, method of preparation and protein concentration are used (Table 4).

The unfractionated oat protein concentrate, rather than an acid extract, was also used to fortify both neutral- and acid-type beverages. Concentrates both with and without water-soluble components were evaluated by a taste panel (Table 5). Scoring was done on an intensity scale, and a descriptive rating score, which usually described existent negative qualities (bitterness, astringency, for example), was also given. Since this research was not directed toward beverage recipe formulation, commercially available products were often used as beverage bases.

Beverages, containing 4% oat protein, made from chocolate- and strawberry-flavored milk shake powdered mixes compared favorably with protein-free controls. The weak chalky mouth-feel associated with the protein-fortified drink can be reduced by decreasing protein concentration. Although the chocolate-flavored product containing 3% protein from the commercial oat flour elicited no objectional taste responses, the panel scores it lower. In this instance, the parent flour had been defatted and the amount of water-soluble components had been reduced.

Adding xanthan gum to the beverages aids suspension of the protein concentrate and gives body to the preparation. If the concentrate contains no gum fraction, the suspended particles tend to settle more. In the shake-type drinks near neutral

Table 3–Solubility of Garland protein concentrates in phosphoric acid solution

	Not defatted[a]			Defatted[b] (1-butanol)		
Solid at start (%)	pH	Nitrogen soluble (%)	Protein dissolved (g/100 ml)	pH	Nitrogen soluble (%)	Protein dissolved (g/100 ml)
1	2.02	60.2	0.46	3.00	70.5	0.65
2	2.22	41.0	0.63			
3	2.31	35.4	0.81	2.05	78.4	2.16
5	1.96	32.7	1.25	2.17	69.3	3.18
7	2.15	26.5	1.42	2.43	51.7	3.33
10	2.13	11.9	0.91	2.83	29.1	2.67

[a] The protein content of nondefatted Garland protein concentrate was 76.7%, dry basis. Phosphoric acid, 0.01M, was used. pH adjustments were made by dropwise addition of 1N or 5N hydrochloric acid.

[b] The protein content of Garland protein concentrate from 1-butanol defatted groats was 91.9%, dry basis. 0.1M phosphoric acid was used in all cases except at pH 3; 0.01M was added.

Table 4–Organoleptic evaluation of protein-fortified beverages with 13% sugar at pH 3

Protein	Phosphate conc (M)	% Protein in beverage	Flavor score[a]	Astringency intensity value[b]
Dal oat conc A from:				
Hexane-defatted groats	0.01	Control	7.3	0
	0.01	0.9	5.6	0.9
	0.01	1.8	4.7	1.1
	0.01	2.7	4.2	1.1
Ethanol-defatted groats	0.01	2.5	5.2	1.0
1-Butanol-defatted groats	0.01	2.5	4.0	1.1
Nondefatted groats	0.04	Control	6.8	0
	0.04	2.0	5.3	0.7
	0.04	2.2	5.2	1.1
	0.06	2.2	4.9[c]	1.5
	0.10	1.6	5.7[c]	1.0
	0.06	Control	5.9[d]	0
	0.06	2.2	5.0[d]	1.4
Garland oat conc A from:				
Hexane-defatted groats	0.04	2.8	4.4[c]	1.6
	0.10	2.4	5.1[c]	1.1
Nondefatted groats	0.01	Control	6.6[c]	0
	0.01	2.0	4.9[c]	1.6
	0.10	Control	6.9[c]	0
	0.10	1.7	5.6[c]	0.9

[a] On a blandness scale from 1 to 10 with 10 being the most bland

[b] On an intensity scale with 1.0 as weak, 2.0 as moderate and 3.0 as strong

[c] With orange flavoring

[d] With carbonation and orange flavoring

Table 5–Organoleptic evaluation of protein-fortified beverages

Protein conc source	Dispersing medium	Protein in beverage (%)	Flavor score[a]	Intensity value[b]
Nondefatted Dal groats flour[c]	Strawberry flavored milk shake mix	4	5.9	1.0 Graininess
	Control (same as above)	None	6.7	
Nondefatted Dal groats flour[c]	Chocolate flavored milk shake mix	4	6.3	0.7 Graininess
	Control (same as above)	None	6.3	
Defatted commercial oat flour[c] (1:6)	Chocolate flavored milk shake mix	3	5.0	0.7 Bitter
Defatted commercial oat flour[c] (1:8)	Water	3	4.7	1.0 Cereal 0.6 Bitter
Nondefatted Dal groats flour[d]	Water	3	4.2	1.0 Bitter
Nondefatted commercial oat flour[e]	Water	3	4.3	0.6 Bitter
Defatted commercial oat flour[e]	0.03M malic acid	2(pH 3.2)	5.0	1.2 Sweet 1.3 Acid-sour
Defatted commercial oat flour[e]	Orange juice	2(pH 3.9)	4.8	0.9 Sweet 0.9 Acid-sour
	Above (control)	None(pH 3.8)	5.2	1.0 Sweet 1.2 Acid-sour

[a] On a blandness scale from 1 to 10 with 10 being the most bland
[b] On an intensity scale with 1.0 as weak, 2.0 as moderate and 3.0 as strong
[c] The protein concentrate A in Figure 1
[d] The protein concentrate B in Figure 2
[e] The protein concentrate C in Figure 2

pH, a combination of around 0.35% xanthan is optimum.

Beverages consisting simply of 3% oat protein blended into water were also tasted by the panel. Neither sweeteners nor flavors were added. The samples were rated the same in flavor intensity even though the concentrate source was different. The drink made with the commercial oats concentrate rated an almost negligible amount of bitterness and was much thicker in consistency than the one containing laboratory-prepared Dal concentrate. The difference in viscosity no doubt relates to the condition of the oat gum fraction in each.

To reduce the high viscosity of an oat concentrate milklike mixture, synthetic beverages of oat concentrate A plus the gum-water-solubles fraction were formulated. The ratio of 1:8, gum-solubles fraction:oat concentrate gave the desired milklike viscosity. In Table 5 appears the tasting scores of an "oat milk" consisting of 1 part soluble fraction with 8 parts of concentrate. The score was higher than that previously mentioned; however, the tasters recorded a cereal, bitter taste. Adaptations of this simple beverage formulation including sweetener and flavor present good possibilities for simple high-protein milklike beverages.

As previously shown in Table 4, acidic-type beverages containing oat protein concentrate possessed weakly astringent properties. These concentrates were produced from laboratory-milled oat groats. If the concentrate was prepared from commercially produced groat flour, acidic beverages could be formulated that possessed a negligible astringency response. Oat concentrate-fortified beverages of this type are listed in Table 5 where either 0.03M malic acid or reconstituted frozen orange juice was the dispersing medium. Sugar (12%) was added to each. The presence or absence of the water soluble-gum fraction in the malic acid-based preparations made little difference in taste panel scoring. Protein-fortified orange juice was scored nearly the same as the orange juice control and exhibited no trace of a cereal taste.

Oat concentrates were also incorporated into carbonated cola-type drinks. The carbonated protein dispersions (1–2%) are delivered from a seltzer bottle as a thick creamy foam, which collapses in time and forms a pleasant tasting product. Concentrates containing the oat gum fraction were favored in such carbonated preparations.

Freeze drying of various types of beverages that contained oat protein concentrate proved to be a useful storage method. For example, laboratory preparations of oat protein concentrate in a strawberry-flavored shake base, in a lemon-flavored ade and in orange juice were freeze dried and kept at room temperature in closed containers. After 6 months' storage at room temperature, the dry powders were constituted with water. The powders dispersed rapidly with stirring and a flavored "oat" beverage was prepared.

Beverages containing 2.4–2.8% protein from Dal concentrate (containing no gum fraction) prepared with commercial shake and ade powders were stored for a year or more at room temperature in sealed containers. The preparations contained 0.1% sodium benzoate and 0.15% xanthan gum. Upon standing, two separate phases were noticed within 1½ hr in some, overnight in others, but were easily redispersed by shaking. Even after 1 yr, no rancid or fermentation odor was detected, no mold had developed and the color had not faded. The pH of these preparations was between 3.5 and 6. Use of a higher concentration of xanthan increased the particle suspension time. After long periods of storage, viscosity decreased.

Other similar beverages made with a protein concentrate from the commercially processed oat flour were also tested for storage life. These were placed in a refrigerator and kept up to 7 months. Each had about 2% of the gum-containing oat concentrate. Two-phase separation occurred as with the Dal concentrate, the color was stable and no sign of mold or spoilage was noted.

Although oat protein-fortified beverages can be stored, it seems perferable to prepare the beverage within a reasonable time before drinking. Such preparations are fresh and less prone to phase separation and bacterial contamination. Other modifications of processing or additives could effectively extend shelf life.

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Ms received 8/14/75; revised 11/1/75; accepted 11/5/75.

We thank K.A. Warner and the Northern Laboratory taste panel for conducting the organoleptic evaluations.

The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

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METHOD FOR EVALUATING THE FILTERABILITY OF WINE AND SIMILAR FLUIDS

ABSTRACT

A method for determination of the filterability of wine was developed. The method is based on evaluation of the filtration curve of a wine filtered through a membrane disc 13 mm in diameter, having a pore size of 0.45 micron. Results of the filterability test were found to be related to plant operation. According to the method developed, a wine is ready for membrane filtration the moment that its filterability test indicates that the differential filtered volume remains unchanged for most of the 60 min determination time. A decrease in the differential filtered volume indicates that the wine contains insolubles/microorganisms, and the rate of the decrease should be considered to be proportional to the load on the membrane. This method is not limited to the wine industry, but can be adapted and/or modified for similar applications, i.e., pharmaceuticals, breweries, fine chemicals.

INTRODUCTION

FILTRATION is an operation, or processing stage, in which insoluble solids are separated mechanically from the fluid in which they are dispersed. The objectives of filtration vary among processes. In the process of winemaking the objective is both to clarify and to stabilize the wine system by the effective removal of insoluble solids and microorganisms.

Traditionally, wines are clarified in a series of steps. Centrifugation, sedimentation and depth filtration may be used to remove the larger particles from wine. However, removal of large particles does not necessarily guarantee a stable wine, i.e., a wine free from turbidity, haze and microbial defects.

Stabilization of wine for bottling is done by one of two methods. One method is heat treatment which may provide protection against microbial defects. The other is membrane filtration which removes microorganisms as well as the visible particles contributing to haze and turbidity (Amerine and Joslyn, 1970; Millipore Corp., 1974).

Stabilization of the wine system by means of filtration may represent: (a) Energy cost savings; (b) Elimination of the questionable heat treatment; and (c) Assurance that the wine will remain clear and free from haze and microbial instabilities—provided that the preceding steps of winemaking have been promptly and properly executed.

Wine to be stabilized by filtration is passed through a membrane having uniform openings. All the insoluble solids and microorganisms that are larger than the absolute pores/openings will be retained on the inlet surface. The emerging filtered wine will practically be stable and ready for bottling.

The retention of insolubles and microorganisms on the inlet surface will result in a gradual blocking of the membrane pores, thereby decreasing the through flow. When the output through the membrane becomes uneconomical and since elevating the pressure drop to maintain the desired flow is risky, the membrane is removed, discarded, and a new one is put into operation. This results in a substantial increase in operational cost.

The objective of a winemaker looking for a high volume membrane filtration is to reduce the load of insoluble solids and microorganisms prior to that step. The most effective way to accomplish that reduction is by capitalizing on depth filtration such as: tight powder filtration and pad filtration, as well as by keeping proper sanitary conditions, minimizing holding time between consecutive stages, and keeping holding temperatures as low as economically possible.

At the present time, the winemaker has no means to evaluate the filterability of a wine i.e., whether a wine is ready for membrane filtration or if it is contaminated and should be refiltered to prevent clogging of the membranes.

The existing Silting Index Method (in which the time required for a measured volume of liquid to pass through a 0.8 micron membrane is taken as an index for the degree of contamination of the liquid) has a limited value in a multiproducts operation. In a large winery, the products are numerous, varying in composition, processing techniques and operating temperatures. This results in a large variation of product and kinematic viscosities. As such, one can expect that different products will exhibit different flow rates through the membrane, thus an index based on one value of total flow rate ($ml/hr/cm^2$) may not be useful to define a degree of contamination, unless the product is seriously contaminated. The 0.8 micron membrane may be inadequate to measure contamination, especially when particles smaller than 0.8 micron may be encountered (Millipore Corp., 1975).

The objective of this work has been to develop means by which the filterability of a wine or a similar liquid can easily be defined.

EXPERIMENTAL

THE SYSTEM for evaluating the filterability of wine (Fig. 1) is constructed from the following parts:

1. Nitrogen pressure tank, with gas pressure regulator and gauges (PI1 and PI2).
2. A bench-top pressure reservoir with an accessory filter holder (such as the 600 ml Filling System, product of Millipore, XX 11 000 00 and their Swinnex 13 mm filter holder, SX 00 013 00).
3. Membrane filter discs 13 mm in diameter to fit filter holder.
4. A graduated cylinder, or semi-analytical electrical top-loading scale.
5. A timer.

To operate the system the outlet pressure of nitrogen is kept at 50 ± 1 psi.

A membrane, 13 mm in diameter, is introduced aseptically into the filter holder. The filter holder is connected to the reservoir. Wine for filterability determination is filled into the pressure reservoir and the graduated cylinder is placed under the filled reservoir. The reservoir is now pressurized through the inlet pressure connecting tube, and the volume of filtrate as a function of time is recorded.

RESULTS

THE OBJECTIVE of this work has been to develop means by which the filterability of wine and similar liquids will be defined. The potential of a wine to plug a membrane has been considered to justify further study beyond the Silting Index.

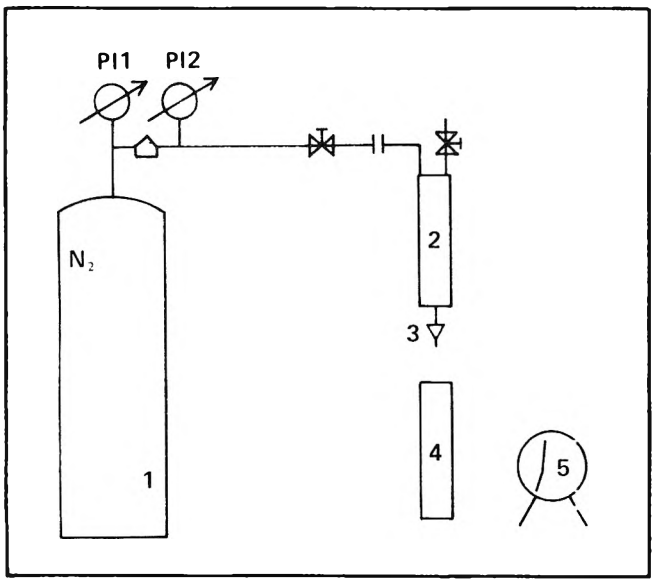

Fig. 1—Schematic diagram (not to scale) of the set up for determination of filterability.

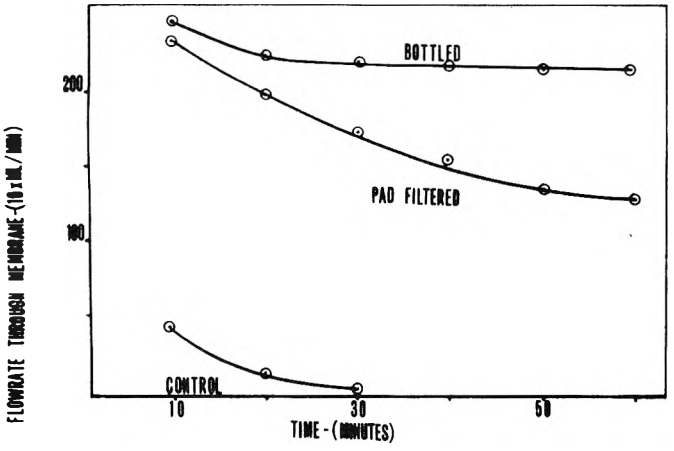

Fig 2—Results of filterability tests conducted on white wine.

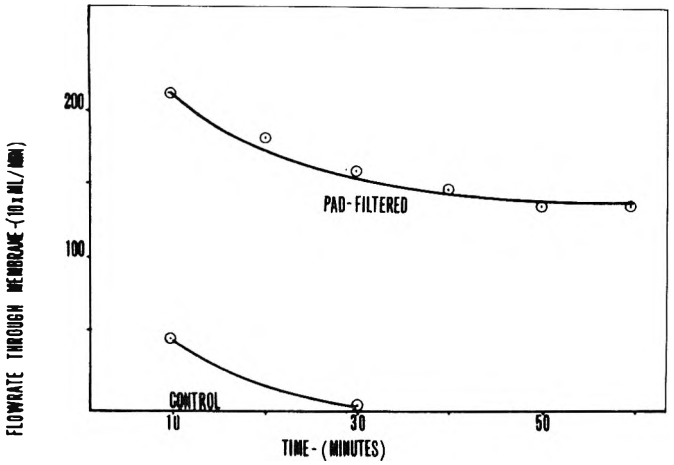

Fig. 3—Results of filterability tests conducted on white wine.

Instead of determining the time required for a measured volume of liquid to pass through a 0.8 micron membrane, the rate of membrane plugging—as determined by the changing rate of filtration—has been considered to be more appropriate.

In the wine industry, the final stabilization of wine is done by filtration through a membrane having pore openings of 0.65 micron. The logical way to determine filterability seems to be, therefore, by the use of a 0.65 micron membrane. However, in order to have results that are highly significant and easily reproducible, a membrane having pore openings of 0.45 micron has been considered and successfully used.

In other industries, where different tolerances for particulates must be enforced, a membrane with different (smaller or larger) pore size will probably be used when this method is adopted.

Two other interrelated considerations have been: minimizing the time required for a single determination, and minimizing the sample size required for a determination. It has been found that both objectives can be achieved by increasing the nitrogen pressure as a driving force for filtration (50 psig) and maximizing the ratio between sample volume to filtration area (use of discs of 13 mm diameter, the smallest available commercially). In industries where different conditions prevail, different pressure and disc diameter may be considered. The flow of wine through a membrane will be a function of:

1. The pressure drop across the membrane (can be kept constant as in the mentioned system).
2. The properties of the wine to be processed (i.e., temperature, composition, and temperature related properties such as viscosity) that are different in each case of wine and operation (later expressed as k).
3. The load of insolubles and microorganisms having larger or equal diameter to 0.45 micron.
4. The number of open pores of the disc.

Once the working pressure and the wine for evaluation have been chosen, the filterability of the wine becomes dependent on the two latter variables, formulated as:

$$F \equiv f(L; E) \tag{1}$$

Where: F is the throughput flow; L is the load of insolubles and microorganisms having a diameter larger than the pore size; and E is the number of open pores. If the contents of the tank have been thoroughly mixed one may consider L to be a constant. E, however, is a function on the volume of wine (V) passing through the membrane. The higher the volume passing through the membrane the greater the decrease in flow rate will be due to plugging of the membrane by the load (L).

One can express this relationship (at constant pressure) mathematically as:

$$-\frac{dV}{dt} = kLV^n \tag{2}$$

and after integration:

$$t = \frac{1}{(n-1)k \cdot L \cdot V^{n-1}} + \text{Constant} \quad n > 1 \tag{3}$$

The objective of this work has not been to define k, L, or n, but by means of the available experimental results, to decide whether a wine is ready for its subsequent processing stage, resulting eventually in membrane filtration. However, the values of the mentioned constants may be determined if so desired by plotting log t vs log V.

To express the filterability of wine, the flow through the membrane was determined every 10 min, and the rate of filtration, $10(\Delta V/\Delta t)$, was plotted vs elapsed time of filtration, t.

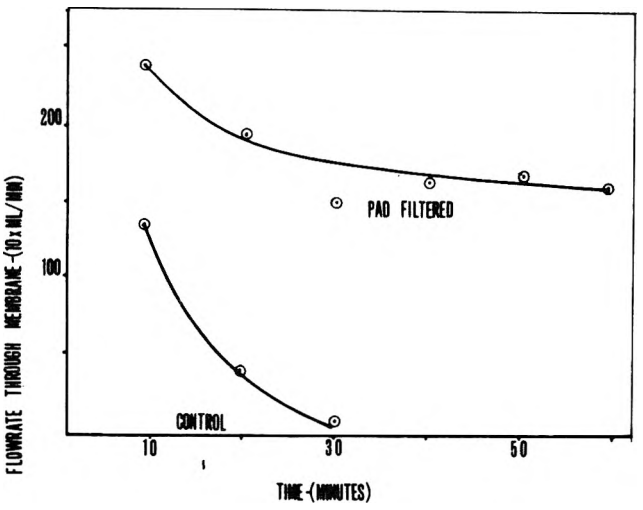

Fig. 4—Results of filterability tests conducted on white wine.

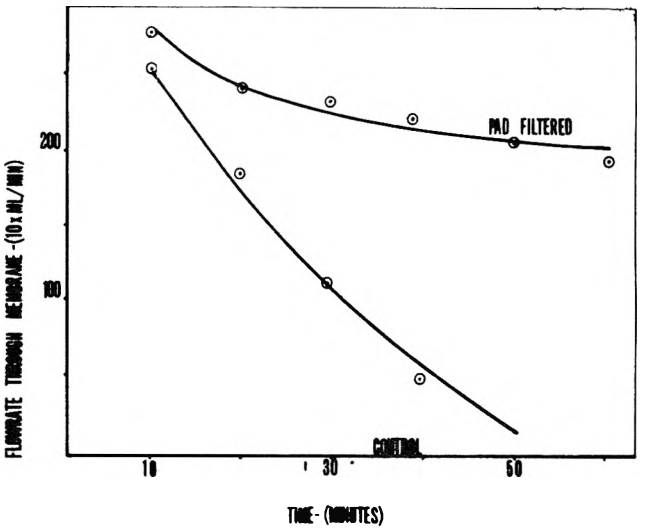

Fig. 5—Results of filterability tests conducted on white wine.

Figure 2 clearly indicates that the filterability of the control wine (a white wine, after racking, rough filtration and 3 months of aging) was very poor. For all practical purposes, plugging of the membrane occurred within 30 min.

When the same wine was subsequently pad-filtered, a significant improvement in filterability was noticed. However, the decreasing flow rate with time through the membrane indicated that the removal of particulates from the wine had not been fully achieved, and as such the wine would still represent a plugging potential to the membranes, due to visible/invisible particulates. It could be seen that the wine bottled after a 0.65 micron membrane filtration/stabilization did not plug the filterability membrane, and should be considered for all practical purposes to be clean.

It is obvious that in each stage of filtration, and especially in the stage preceding membrane filtration the winemaker must strive to get a wine having a constant filterability through the membrane, since it will mean a clean and stable wine with a minimal risk to plug the membrane. When the filterability of the wine is decreasing, the wine represents a risk to the membrane.

The results given in Figure 3 represent a different pad filtration preformed on the same control wine as in Figure 2. The filterability curve was smoother, more closely resembling the desired curve. In practice and actual plant operation these two curves represented a more than threefold difference in membrane life: 8,800 gal were passed through the 0.65 micron membrane cartridge with the pad filtered wine from Figure 3 as opposed to 2,700 gal of wine in the first case of the pad filtered wine from Figure 2.

The advantage resulting from executing filtrations promptly and properly can be seen by comparing Figures 4 and 5. The control wine from Figure 5 received a powder filtration prior to pad filtration while the control wine from Figure 4 did not. Powder filtration improved the filterability of the control wine significantly, resulting later in improved filterability of the pad filtered wine (Fig. 5). The final improvement as seen in plant operation was noticed when more than 20,000 gal of wine passed through the membrane cartridge. The pad filtered wine had almost the same filterability as the membrane filtered wine in Figure 2.

If the Silting Index had been used on the control wines of Figures 4 and 5, the plugging potential of these wines may have been missed as these wines are fairly clean as compared to the control wines of Figures 2 and 3.

The application of the filterability test for evaluation of equipment is given in Figures 6 and 7. The operation of a Pressure Leaf Filter was studied. The responses measured included: flow rates, pressure drops, turbidity and filterability as a function of the on going filtration cycle. While flow rate remained fairly constant and the pressure drop was building, one could notice that the low turbidity level was established after 20 min and remained constant. However, with increasing time into the filtration cycle, the filterability of the filtrate improved steadily indicating a further reduction in the "invisible" load. As such, longer cycles were not only justified economically, but also from the quality and stability point of view. The comparison of turbidity data and filterabilities shows the big advantage of the filterability method. A turbidimeter can detect only those particles with a refractive index much different than the medium in which they are dispersed, while the filterability will be influenced by particles having different and like refractive indices than the medium. As such, a turbidimeter may tell us that a wine is not turbid, but it will not caution the winemaker of any microbial load unless it is very high. In some cases, as when Port wine was filtered with the Pressure Leaf Filter, the filtrate coming out later in the cycle could be considered even sterile (Fig. 8).

Another case study involved a wine made from six components which was found later to plug membranes in the bottling operation. The reason for plugging was traced. The filterability of the blend was studied and found to be poor, essentially 0 as can be seen in Figure 9.

Table 1—Results of filterability of wines differential volume filtered—of six blend components

Time (min)	Component					
	A	B	C	D	E	F
10	15	10	10	50	105	–
20	–	–	–	15	40	–
30	–	–	–	5	20	–
40	–	–	–	–	–	–
50	–	–	–	–	–	–
60	–	–	–	–	–	–

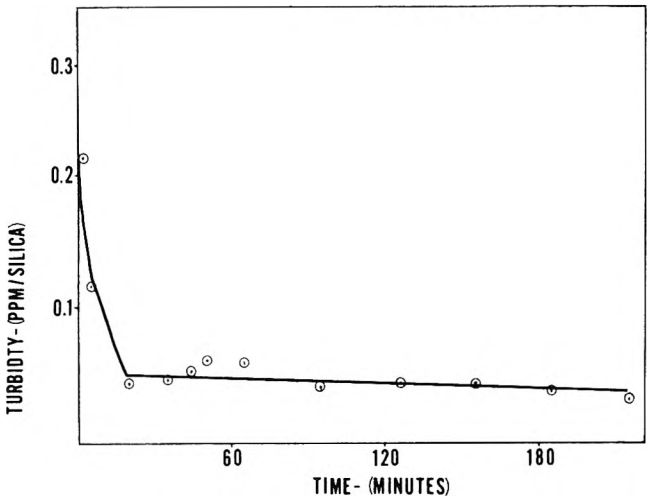

Fig. 6—Turbidity vs elapsed filtration time of wine through pressure leaf filter.

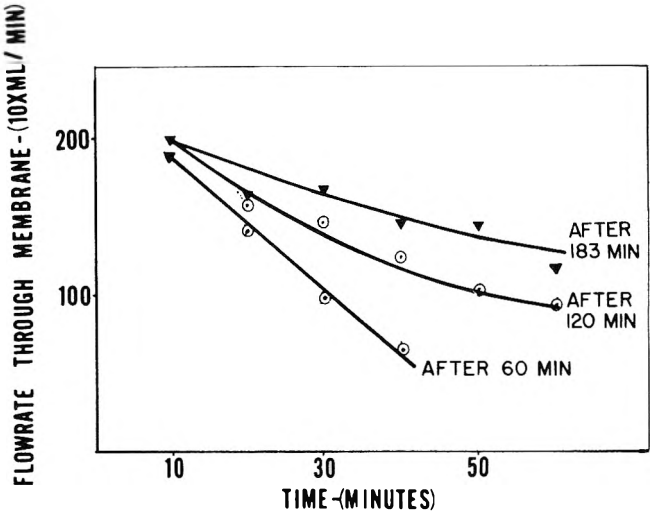

Fig. 7—Filterability of wine through pressure leaf filter.

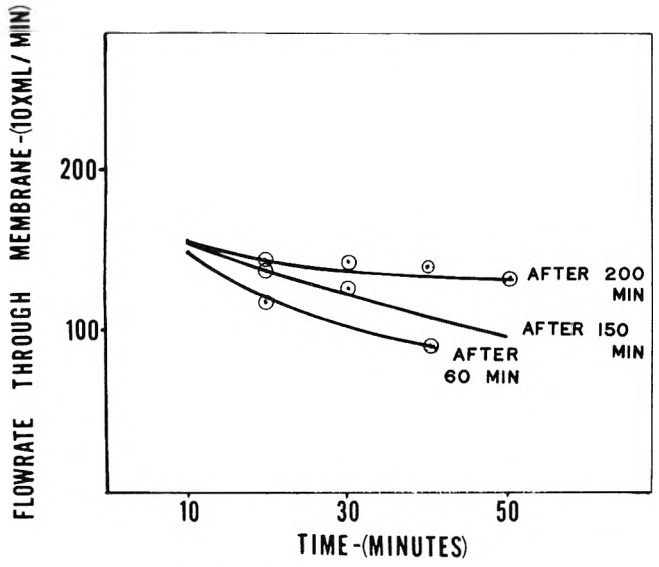

Fig. 8—Filterability of Port wine.

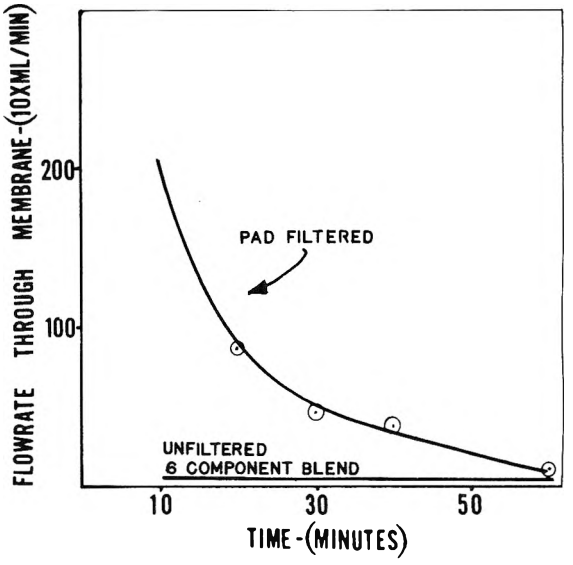

Fig. 9—Filterability of a blend of red wine after a pad filtration.

When the blend was pad filtered, its filterability improved significantly; however, it was still unsatisfactory for stabilization. This was noticed later by frequent plugging of the membranes. At that time it was of interest to find out whether all the components in the blend were loaded with particulates. As can be seen from Table 1, all the components represented heavily loaded wines that will require further filtration after blending and prior to pad filtration. In addition determination of filterability was sensitive enough to determine the increase in through flow of a wine when its temperature was raised, and it was found to be sensitive enough to define a significant decrease in filterability of wine kept overnight at 76°F.

CONCLUSION

A SIMPLE METHOD for determination of filterability of wine and similar liquids has been developed. The method, in a matter of 60 min, can give information on wine stability and whether a wine is ready for subsequent processing and/or bottling. The results of filterabilities obtained by the method should not be used on an absolute basis as they are a function of the composition, physical properties and temperature of the wine under consideration.

If so desired, the disc retained in the membrane holder can be used for further studies [such as microbiological (Kunkee and Neradt, 1974), etc.] to establish the cause of the membrane plugging.

This method is not limited to the wine industry, but can be adapted and/or modified for similar applications, i.e., pharmaceuticals, breweries, fine chemicals.

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Ms received 8/20/75; revised 11/29/75; accepted 12/2/75.

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ABSCISSION AGENT EFFECTS ON ORANGE JUICE FLAVOR

ABSTRACT

The flavor quality of juice products from early- (Hamlin), mid- (Pineapple) and late-season (Valencia) oranges sprayed with abscission chemicals to aid in mechanical harvesting, was evaluated and compared with that of similar juice products from untreated fruit. Four commercially available abscission agents were included in this study: Acti Aid®, Release®, Pik-Off® and ethephon. An expert taste panel distinguished experimental from control juice samples in most tests and generally preferred juice products made from control oranges.

INTRODUCTION

MOST OF the Florida citrus fruit crop can be harvested mechanically because it goes into processed products. The anticipated expansion of mechanical harvesting of oranges within the Florida citrus industry introduces a need to assess the effects of mechanical harvesting on product quality. In most feasible mechanical harvesting systems being tested, application of abscission chemicals is required to loosen the fruit prior to mechanical harvesting. Chemicals function by altering metabolic pathways within the fruit and could thus affect formation or balance of flavor components. To date no systematic study to evaluate the effects these chemicals may have on flavor quality of citrus products has been reported.

The need for abscission agents to effectively harvest citrus mechanically was reported by Cooper et al. (1968) and Wilson (1969). Later, Cooper et al. (1969) reported on the exceptional potency of cycloheximide (Acti Aid) as an abscission agent for citrus. Although cycloheximide can be used successfully for the early- and mid-season fruit, it is not suitable for late-season (Valencia) fruit because at harvest time the trees are carrying young fruit of the next season's crop and fruitlet drop could be excessive (Cooper and Henry, 1973). In 1973, Wilson reported on the abscission activity of 5-chloro-3-methyl-4-nitro-1H-pyrazol (Release). Tests by Kenney et al. (1974) demonstrated the effectiveness of Release as an abscission agent for citrus. Release loosens late-season Valencia matured oranges without damage to the young fruit of the next season's crop.

With full Federal clearance for use of Acti Aid, clearance for experimental use of Release and clearance requested for glyoxal dioxime (Pik-Off), a promising abscission agent effective for mature Valencia oranges, (Wilcox et al., 1974) the use of abscission agents is becoming more widely accepted.

The three agents mentioned act through damage to the peel which causes the release of wound ethylene thus promoting abscission. A fourth compound, ethephon, was used to compare a non-rind injuring abscission agent with the three rind-injuring chemicals. Other metabolic changes also occur that could have an effect on the flavor quality of products made from the treated fruit. Because of the changes in metabolic pathways, abscission agents might have effects which vary with cultivar, maturity and season. Thus, a study was undertaken to determine the effects of these abscission agents on the flavor quality of processed juice products obtained from early- (Hamlin), mid- (Pineapple) and late-season (Valencia) oranges at different stages of maturity. The four abscission chemicals employed were those most commonly used in experimental and limited commercial trials by the citrus industry.

EXPERIMENTAL

EXPERIMENTAL ORANGES sprayed with abscission agents and unsprayed control oranges were obtained from trees with similar root stock and selected from the same area of a grove, that had been irrigated, fertilized and sprayed the same way. The variety and maturity of the oranges, the type and concentration of abscission agent, the date of harvest, the brix/acid ratio and the length of time between spray and harvest are shown in Tables 1 and 2.

Juice samples

Single strength. Experimental and control orange juice samples were prepared by identical procedures. Oranges were thoroughly washed and processed with a commercial FMC In-Line Extractor with a 7/16 in. longbore orifice tube and a 3/4 in. down beam setting. The juice was passed through a pressure-screen finisher with 0.033 in. diameter holes to remove seeds and excess pulp; it was then pasteurized and sealed in 46-oz cans and stored at −18°C until used.

Concentrate. Experimental and control processed single-strength orange juice samples were concentrated by using a rotary evaporator. Samples weighing 1100g were concentrated to 250–300g (approx 40° Brix) at a pressure of 1.5–3.0 mm Hg with the temperature held below 45°C. For reconstitution to single-strength for taste evaluation the concentrate was placed into a 2-liter beaker on a magnetic stirrer. Orange oil, obtained from control oranges of the same variety and maturity was added dropwise to restore the oil content to 0.02% by volume. The stirring was continued for 15 min after the oil was added, then water was added to bring the sample back to its original weight.

Storage study

Processed canned single-strength orange juice sample numbers 1, 2, 5 and 6 listed in Table 1 and samples 1 and 2 in Table 2 were thawed and placed in laboratory lockers at 21° and 30°C. These juices were compared weekly with identical juice stored at −18°C for determination of the storage time at which a threshold flavor change would be detectable by an expert panel.

Flavor evaluations

The triangle and paired comparison tests employed were discussed by Boggs and Hanson (1949). For triangle and paired comparison tests, 12 experienced tasters were each given two presentations. In triangle tests, there were three samples per presentation, two of which were identical. Judges were asked to indicate which sample had the different flavor. In paired comparison tests judges were asked to indicate which sample they preferred. These tests compared: (1) processed single-strength experimental (abscission-treated) orange juices vs equivalent control (nontreated) juices; (2) experimental orange juice concentrates vs equivalent control concentrates; or (3) stored experimental and control processed single-strength orange juices held at 21° and 30°C vs the identical juices held at −18°C. The latter samples were tested at 7-day intervals.

RESULTS & DISCUSSION

THE TASTE PANEL distinguished most experimental abscission-treated from control nontreated samples and generally preferred the control juice. Juice of barely-matured and well-matured fruit of each of the three cultivars listed in Tables 1

Table 1—Flavor evaluation of juice from early- (Hamlin) and mid-season (Pineapple) abscission chemically treated and control oranges

Sample no.	Harvest date	°Brix/acid ratio	Abscission agent	Spray concentration of agent (ppm)	Days on tree after spray	Flavor evaluation confidence level: Difference exp vs control	Flavor evaluation confidence level: Preference for control
			Hamlin oranges				
1	11-25-73	10.45	Acti Aid	20	7	0.05	NS[a]
2	01-04-74	12.86	Acti Aid	20	7	0.001	0.01
			Pineapple oranges				
3	01-25-74	9.87	Acti Aid	20	6	0.001[b]	0.01[b]
4	03-21-74	17.13	Acti Aid	20	6	0.001	0.01
5	01-17-75	12.22	Acti Aid	10	6	0.001[b]	0.01[c]
6	03-11-75	13.95	Acti Aid	10	6	0.001	0.01
7	01-17-75	13.22	Ethephon	250	6	0.01	NS

[a] Not significant at 0.05 confidence level or greater
[b] For both single-strength juice and concentrate
[c] Not significant for concentrate

and 2 was included in the test. Concentrations of chemicals applied and days oranges were left on the trees after spray were recommended by Cooper (1975) as being typical of conditions of commercial harvesting.

Because the ongoing metabolic processes are affected by the abscission chemicals, it is possible for the effects on flavor to vary depending on the maturity of the fruit sprayed. Abscission agents may cause a further aging of well-matured fruit with adverse effects on flavor quality. The flavor compounds responsible for the flavor change have not yet been isolated and could also include a partial flavor effect from the abscission agents themselves. As outlined in Tables 1 and 2, samples of barely-matured and well-matured Hamlin, Pineapple and Valencia oranges were treated with 20 ppm Acti Aid. Since Pineapple oranges have been reported to require less abscission chemicals, (Cooper and Henry, 1973) samples sprayed with 10 ppm Acti Aid were also evaluated for this cultivar. The taste panel detected a difference in all processed single-strength juice samples from oranges sprayed with Acti Aid when the same sample was compared with that from unsprayed control fruit of the same cultivar and maturity. Experimental samples were detectable at a 0.001% confidence level except for those from barely-matured Hamlins which were detected at a 0.05% level, and well-matured Valencia at a 0.01% confidence level. The low sugar content of early Hamlin and the high sugar content of late Valencia fruit probably accounted for the lower confidence levels of these two samples. In the paired comparison tests, the panel showed a preference at the 0.01% confidence level for juice from the control fruit with the exception of juice from barely-matured Hamlin oranges for which there was no preference.

A difference shown in Table 2 between control and experimental Valencia juice sprayed with Release at 250 ppm was detected in barely- and well-matured samples at the 0.001% confidence level; the paired comparison test showed a preference for control juice in both tests at the 0.01% confidence level. Flavor tests were also run on juice from Valencia oranges sprayed with only 150 ppm Release and left on the tree for 3

Table 2—Flavor evaluation of juice from late-season (Valencia) abscission chemically treated and control oranges

Sample no.	Harvest date	°Brix/acid ratio	Abscission agent	Spray concentration of agent (ppm)	Days on tree after spray	Flavor evaluation confidence level: Difference exp vs control	Flavor evaluation confidence level: Preference for control
1	3-28-74	13.77	Acti Aid	20	7	0.001	0.01
2	6-24-74	15.94	Acti Aid	20	7	0.01	0.01
3	4-02-75	10.44	Release	250	5	0.001	0.01
4	6-03-75	12.02	Release	250	4	0.001	0.01
5	3-25-75	9.70	Release	150	3	0.001	—[a]
6	3-27-75	9.56	Release	150	5	0.001	0.01
7	4-02-75	10.61	Pik-Off	300	5	0.001	0.01
8	6-03-75	11.61	Pik-Off	300	4	0.001	NS[b]
9	3-25-75	10.75	Pik-Off	300	3	NS	—[a]
10	3-28-75	10.01	Pik-Off	300	6	0.001	0.01

[a] Test not run.
[b] Not significant at 0.05 confidence level or greater.

and 5 days. The concentration of the agent and number of days the fruit were left on the tree were reduced to determine flavor effects of Release even before the fruit had loosened adequately for harvesting. Flavor difference and preference of the juices in the 5-day test after spraying with 150 ppm Release was the same as for the juices evaluated after spraying with 250 ppm Release. The preference test for juice from oranges left on the tree only 3 days was not run because the fruit had not loosened enough for mechanical harvesting.

Triangle flavor tests of Valencia orange juice made from oranges sprayed with 300 ppm Pik-Off, showed a difference in flavor at a 0.001% confidence level when treated fruit was left on the tree for 4, 5 or 6 days. Juice from oranges sprayed with Pik-Off and left on the tree for only 3 days to determine the effects before fruit was adequately loosened for harvest, showed no significant difference in flavor when compared with juice from unsprayed control fruit. In those tests where differences were detected, paired comparison tests of Valencias sprayed with Pik-Off show preference for control juices at the 0.01% confidence level for all but the well-matured Valencia juice for which the panel indicated no preference. It appears that the high degree of sweetness of the late-Valencia juice masked any effect of the abscission agent.

A single test was run by using 2-chloroethylphosphonic acid (ethephon) on barely-matured Pineapple oranges. This test was conducted because ethephon enters, breaks down and releases ethylene needed for abscission near the cite of abscission while the other abscission agents injure the fruit and cause production of ethylene (Cooper and Henry, 1973). Triangle taste testing of the processed juice showed a difference in flavor detected at the 0.01% level of confidence, but the paired comparison test showed no preference when juice was compared with that of unsprayed control juice. This indicates that an abscission agent that damages the peel has a more adverse affect on flavor quality than an abscission agent (ethephon) that only releases ethylene. However, ethephon causes excessive leaf drop, making it an undesirable abscission agent for use with mechanical harvesting.

As concentrate is the major commercial orange juice product produced, a test was made to determine whether volatile constituents that would be removed during concentration were causing flavor changes. Orange juice concentrate made from experimental juice obtained from Pineapple oranges sprayed with either 10 or 20 ppm Acti Aid and from similar control juices was reconstituted and compared for flavor differences and preferences (samples 3 and 5 of Table 1). Table 1 shows that after both levels of spraying the panel was able to detect a difference in flavor of both reconstituted juices. The paired comparison tests showed no preference between control and experimental juice from oranges sprayed with 10 ppm Acti Aid but a preference was found at the 0.01% confidence level for the control juice when compared with juice from oranges sprayed with 20 ppm Acti Aid. Differences in flavor of concentrates made from control and experimental juices indicate the compounds responsible for the flavor change include nonvolatile components not removed by distillation. Thus, there was no preference for control or experimental reconstituted juice when the recommended 10 ppm Acti Aid was used, but a significant preference for control juice was observed when an excess (20 ppm) of Acti Aid was applied.

In order to determine whether the use of abscission chemicals affected the rate of flavor change of juices during storage, studies were conducted at 21° and 30°C with processed single-strength juices from Hamlin, Pineapple and Valencia oranges. Triangle flavor tests showed that flavor changes occurred at the same rate in experimental and control juices when these were compared with juices from the same batch kept at 0°C. Hamlin and Pineapple juice flavor changes occurred after 1 wk at 30°C and 2 wk at 21°C. Valencia juice flavor changes occurred after 2 wk at 30°C and 3 wk at 21°C.

Juice products from Hamlin, Pineapple and Valencia experimental oranges treated with any of the abscission agents were distinguished from nontreated samples by the panel. Many of the panel members indicated that the experimental juices had a slightly over-ripe flavor which was considered an adverse effect. In most tests the panel preferred the control juice; no preference was established in a few comparisons of either very acidic or very sweet samples whose flavor may have been strongly influenced by the extreme sour or sweet conditions. However, in no test was the experimental juice preferred by the panel.

Since these results were tested for detectable differences by a trained, experienced panel, it is uncertain whether the general consumer would detect flavor changes in these products or whether such threshold flavor changes would be objectionable. In order to resolve this uncertainty, flavor tests need to be conducted with large untrained consumer panels. At present only a very small percentage of the total orange crop is mechanically harvested and thus, in normal commercial operations this fruit would be mixed with hand-harvested fruit. At current proportions the flavor effects of the abscission agents on the resultant juice blends would be negligible. However, this study emphasizes the importance of minimizing flavor changes by carefully controlling the concentration of these abscission chemicals and of harvesting the fruit as quickly as possible after the spraying has loosened the fruit. Such measures will become increasingly important in the future as mechanical harvesting is more widely adopted.

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Ms received 11/1/75; revised 12/13/75; accepted 12/16/75.

The authors thank Dr. W.C. Cooper and Mr. Frank Galena of the U.S. Horticultural Field Station, Orlando, Fla., for locating and spraying of all fruit samples. The authors also thank Mr. Jack L. Brown of our laboratory for his contribution in harvesting and processing of these samples.

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CLOUD STABILITY TEST FOR PASTEURIZED CITRUS JUICES

ABSTRACT

A 24-hr storage test is presented for evaluating the cloud stability of pasteurized citrus juices. Enzymatic flocculation is accelerated by the addition of a special pectin and a barium salt while controlling pH and temperature. A citrus juice which retains greater than 60% of its initial cloud after 24 hr of this storage will have a satisfactory cloud stability for at least 1 yr under normal conditions.

INTRODUCTION

CLOUD LOSS in citrus juice has long been associated with enzymatic degradation (primarily pectin-methylesterase) of soluble pectin (Cruess, 1914). Commercial citrus processors generally inactivate these enzymes with high temperature-short time pasteurization (Stevens, 1940a, b). This treatment is kept to a minimum as high temperatures tend to impart a "cooked" off-flavor to juice. Operating at minimum temperatures creates a need for a method of testing juice to insure adequate enzyme denaturation.

The activity of pectin-methylesterase (PME) can be chemically monitored by analysis for either the methanol or acid groups that are released as shown in Figure 1. Reports have appeared on gas-liquid chromatographic (Lee and Wiley, 1970; Bartolome and Hoff, 1972; Krop et al., 1974) and spectrometric (Wood and Siddigni, 1971) determination of the released methanol but these methods require as much as 2 hr of operator time and provide questionable results at the low PME levels, around 5×10^{-6} PEu (meq/min/ml), encountered in pasteurized citrus juice. The PME-mediated release of acid groups has been followed by numerous pH methods (Somogyi and Romani, 1964; Vas et al., 1967; Mayorga and Roly, 1971). All these methods have lacked reliability at the very low PME levels which can still cause cloud loss after a few months juice storage.

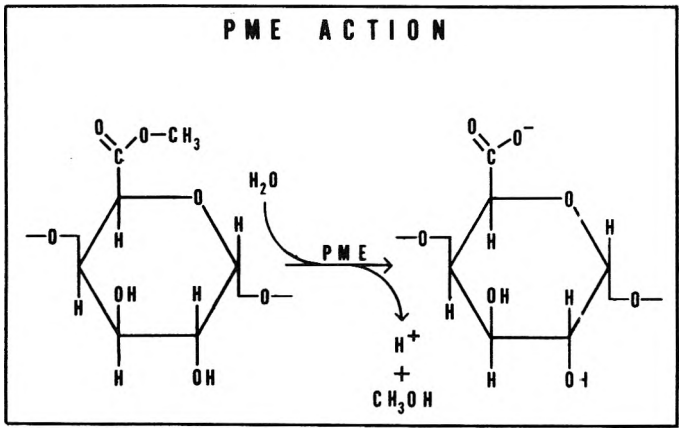


Fig. 1—Reaction mediated by pectin methylesterase.

Two methods of analysis for residual PME activity based on the physical characteristics of stored juice samples have appeared. Rothschild et al. (1975) determined results by the change in stored juice viscosity in the presence of $CaCl_2$ and 55% esterified pectin at pH 7. Stevens (1940b) determined the amount of juice cloud remaining after incubation with a partially demethylated pectin and an accelerating cation at an appropriate temperature and pH for cloud loss acceleration. Pectin degrading enzymes, if present, act rapidly on the added pectin. Both these tests take many days for determination of analytical results at the levels of enzymatic activity encountered in pasteurized juice. They also are "go or no-go" tests which give little information about juices of intermediate stability.

The following project was undertaken to investigate the interaction of the various parameters in the Stevens' 5-day storage test. The aim of the investigation was development of a more rapid, yet reliable test for residual, cloud reducing enzyme activity in pasteurized citrus juice which would be suitable for control use.

MATERIALS & METHODS

THE INGREDIENTS used in both the 24-hr and 5-day tests are listed in Table 1. Tables 2 and 3 list the test formulae used for specific citrus types. Add each ingredient in the order given with thorough mixing. Place final mixture in clean, 1-oz, screw-cap bottles and incubate at 49°C. After 24 hr (or 5 days) remove bottle from incubator, gently invert it three times, then dilute the contents in the ratio, seven parts test solution and nine parts water. Centrifuge the dilute solution at 900 X G for 2 min. Decant supernatant into 0.5-in. spectrophotometer tubes and read at 660 nm vs distilled water in Bausch and Lomb Spectronic 20. Juice sample is "stable" if this reading is $\leq$ 32% T.

Several of the test parameters were held constant in all tests. Juice content and dilution factors gave a readable cloud density. Sodium benzoate content was sufficient to protect mixtures against microbial activity. Pectin was added to a level ensuring excess substrate for enzymic action without causing gelation of mixtures. Time and force of centrifugation was sufficient to remove floc while leaving remaining cloud in supernatent.

Variations of the other test parameters, pectin type, Ba^{++} concentration, pH and incubation temperature, were evaluated by determining the rate of flocculation of a test mixture with a juice containing maximum acceptable enzyme activity for long term juice storage.

The set time of pectins used in this study was determined by a Sunkist internal method (Joseph and Baier, 1949). This method is based on the time required for a standard jelly mix to gel as it is cooled. These times can vary from 10 sec to >5 min with acid retarded pectins.

RESULTS & DISCUSSION

PECTIN used in the cloud test has a major effect on flocculation rate, but good correlation of flocculation rate to pectin characteristics (viscosity, AGA, AGE, MW) was not evident when various lots of commercially produced pectin were checked. Although a general trend of faster flocculation rates

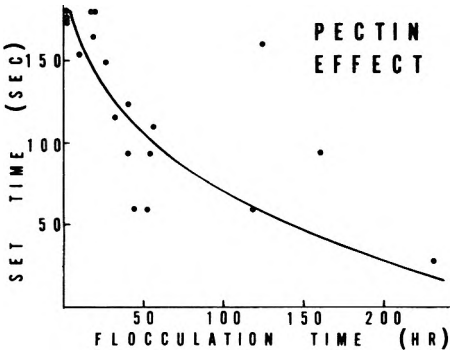


Fig. 2–Effect of commercial pectin set times on time to reach 50% T in accelerated cloud test.

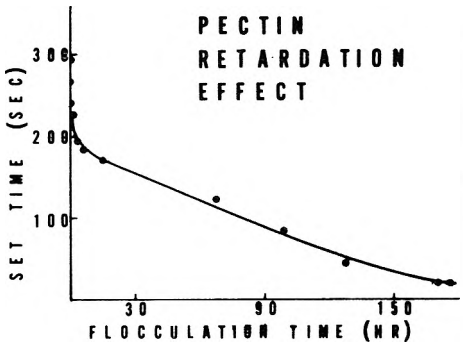


Fig. 3–Effect of the acid retardation of a pectin on time to reach 50% T in accelerated cloud test.

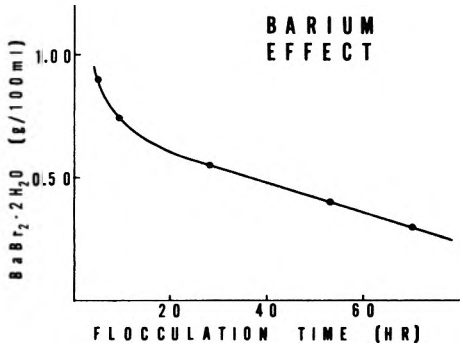


Fig. 4–Effect of barium content on time to reach 32% T in accelerated storage test.

with longer set time pectins was noted there were several exceptions (Fig. 2). Correlation between flocculation rate and set time was good when pectins of various set times were produced from a single rapid set pectin by acid retardation treatments, however. This correlation is shown in Figure 3.

The pectin chosen for the 24-hr cloud test had a set time of 150 sec and gave a 30% T reading in 24 hr on a juice which was borderline stable by the standard 5-day test (unstable in 6 days).

Flocculation rate increased as a test solution's barium content increased as shown in Figure 4. Although change was quite rapid at low barium concentrations, it leveled off considerably between 0.90 and 1.10g $BaBr_2$ dependent upon temperature and pH. At much higher levels, above 1.5g $BaBr_2$/test, nonenzymatic flocculation occurred. In fact, even boiled juice flocculated very rapidly. Although the major inflection varied widely in terms of barium content and flocculation time, all pectins tested gave similarly shaped curves.

The mechanism of Ba^{++} enhancement is unknown, but its ability to cause flocculation in the absence of enzyme activity indicated interaction with the pectin substrate. This proposal was further substantiated by the change in the range of test response to added barium with changes in pectin used. For example, when pectin set time was changed from 28 sec to 2.5 min (an increase in free acid groups), the inflection point in the graph of $BaBr_2$ concentration to time of flocculation changed from about 0.55 to 1.00g $BaBr_2$ per 100 ml test mixture. Also a change in the amount of enzyme activity produced little or no change in this inflection point.

The observed interrelated effects of pH and incubation temperature on flocculation rate are shown in Figure 5. As test solution pH was lowered, temperature had an "on-off" effect on the flocculation rate. At 59°C, flocculation ceased below pH 3.2. Incubation at 49°C allowed a reduction of test solution pH to about 3.0 before the flocculation rate became immeasurably slow. A further reduction in pH was possible at 38°C. As reduced pH and increased holding time both allow a reduction in the temperature requirements for cloud stabilization (Tressler and Joslyn, 1954), the above "on-off" phenomenon may have been due to enzyme denaturation. Thus, at low pH and a holding time of many hours, the incubation temperature may have been sufficient to at least temporarily alter enzyme structure enough for inactivation.

Since Somogyi and Romani (1964) report the pH optimum for citrus PME is about 7.5, an increase in flocculation rate was expected with an increase in pH. This expectation was not realized, however, due to the effect of the cations added in pH adjustment. The net result was instead a decrease in flocculation rate as illustrated for sodium in Figure 6. Note that a test solution, containing juice of borderline stability, will give a stable reading if it is high in sodium content. Sodium contents of up to 0.03M make little difference in test results.

Table 1–Ingredients used in accelerated citrus juice cloud tests

Ingredient	Conc (w/v in H_2O)
Citric acid soln	50% citric acid + 0.10% benzoic acid
Benzoate soln	23% sodium benzoate
Barium salt soln	25% $BaBr_2 \cdot 2H_2O$ or 19% $BaCl_2 \cdot H_2O$
Pectin soln[a,b]	2.75% pectin + 0.15% benzoic acid
$Ba(OH)_2$ "milk"	2.00% $Ba(OH)_2 \cdot 8H_2O$

[a] Special retarded pectins as noted elsewhere, 2.5 min set time for 24 hr test and 1 min set time for 5 day test.
[b] Allow this solution to stand 2 hr before use.

Table 2–Formula for accelerated orange, grapefruit and tangerine juice cloud tests

Ingredient	Quantity
Juice[a]	93 ml
Citric acid soln	Sufficient to give pH 3.15–3.20[b]
Benzoate soln	1 ml
Pectin soln	4 ml
Barium salt soln	4 ml (2.4 ml)[c]

[a] Single strength: 11.8°B, orange; 10.2°B, grapefruit; 11.5°B, tangerine
[b] This can be accomplished by titration or measured addition to the following total contents of citric acid: orange, 2.00g; grapefruit, 1.65g; tangerine, 1.80g.
[c] The lower quantity of Ba^{++} is for the 5-day test.

Table 3–Formula for accelerated lemon juice cloud test

Ingredient	Quantity
Juice[a]	49 ml
Benzoate soln	1 ml
Pectin soln	4 ml
Barium hydroxide "milk"	50 ml

[a] Single strength: 57g citric acid/liter.

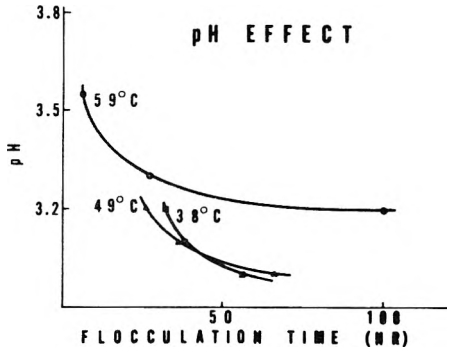


Fig. 5—Effect of pH on time to reach 50% T in the accelerated storage test at three temperatures.

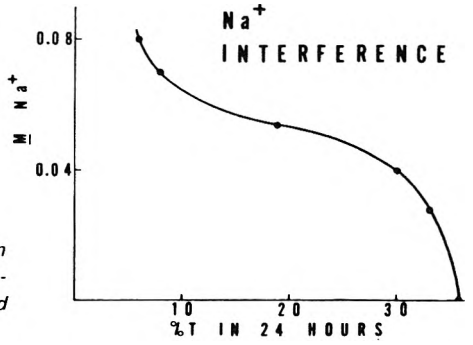


Fig. 6—Effect of sodium ion concentration on flocculation rate in the accelerated storage test.

Sodium, other monovalent cations and most divalent cations (other than barium) are not actually inhibitors of flocculation, but rather enhancers which are much less effective than barium (Stevens and Pritchett, 1938). Other cations probably compete with barium for active sites in the pectin-enzyme system and cause a reduction in the rate of flocculation. The solubility of barium salts preclude the use of barium hydroxide for adjusting the pH of citrus juice test solutions upward to pH 7.

With variations in pH, barium content, pectin and incubation temperature, any number of cloud tests producing results in any reasonable length of time can be developed. With tests producing results very quickly (<8 hr) sensitivity was so great that small changes in enzyme activity caused large changes in flocculation rate. Thus, test results showed all or no flocculation. These highly sensitive tests could not resolve intermediate enzyme levels and a moderately stable juice, suitable for some applications, would be rejected as unstable. Conversely, test solutions having very slow flocculation rates (>5 days) were not very sensitive and the time lag between juice pasteurization and test result was inconvenient. With considerations of accuracy and convenience, the data in the foregoing figures were used to select the test parameters shown in the methods section. This test gave reproducible results over a wide range of residual enzyme activities with orange, grapefruit and tangerine juices.

The rate of flocculation for a just stable juice by the 24-hr test is shown in Figure 7. Note in the region from about 18–45 hr storage that the increase in % T with time is a linear function. Use of this graph allows accurate readings to be taken a few hours on either side of the 24-hr incubation period. Further, this graph is typical of the flocculation patterns of all juices and illustrates how a totally unstable juice can be differentiated from a partially stable or stable juice. In 24 hr an unstable juice would show >85% T while a partially stable juice would show between 40 and 70% T.

Figure 8 compares 24-hr cloud test results with the rate of cloud loss in single strength juices stored at 16 and 38°C. The differences in shelf stability of juices giving different results in the 24-hr cloud test appear to give good correlation. Storage data and repeated cross checks with the standard 5-day cloud test determined that juice showing 30–32% T in the 24-hr cloud test (less than 60% of initial cloud lost) contained the maximum residual enzyme activity for a commercially stable juice.

Lemon juice required a special adaptation of the cloud test, due to low pH. In this test the only changes are in quantity of juice (based on acid content), barium hydroxide in place of a barium salt and point of stability cut off at 50% of initial cloud remaining. The changes in juice quantity and barium form were necessary because of the interference caused by cations other than Ba^{++} and the insolubility of barium citrate. As lemon juice has relatively low and variable cloud content and a dilute test solution was necessary due to citric acid content, results were determined on the basis of percent initial cloud.

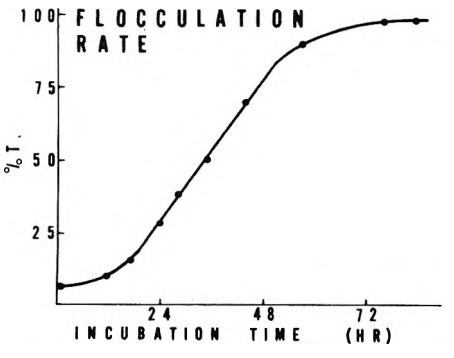
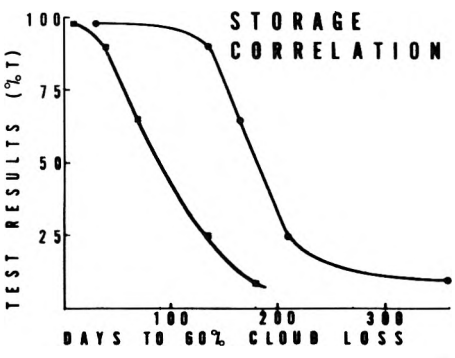


Fig. 7—Decrease in cloud with time of a just stable juice in the accelerated storage test.

Fig. 8—Correlation of shelf storage stability with accelerated test results at two temperatures: ■ 38°C; ● 16°C.



Although the 24-hr test here reported required 1 day of storage before results could be read, technician time per sample was only a few minutes making the test feasible for control use.

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Ms received 9/6/75; revised 12/19/75; accepted 12/25/75.

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LOSSES IN AVAILABLE LYSINE DURING THERMAL PROCESSING OF SOY PROTEIN MODEL SYSTEMS

ABSTRACT

This study was conducted in order to develop a mathematical model for predicting lysine losses during processing of soy products. Model systems used in this study consisted of microcrystalline cellulose, glucose, sucrose, potato starch and soy protein. System composition, water activity, pH and time and temperature of heating were varied and available lysine was monitored by the fluoro-2,4-dinitro benzene method of Carpenter. In the first set of experiments, statistical analysis of the data obtained resulted in an equation which shows the effects of the variables used on available lysine retention: Fraction available lysine remaining = 0.58 + 0.047 pH − 0.093 glucose − 0.059 temperature − 0.0068 time + 0.031 (water activity)^2 + 0.025 a_w · sucrose + 0.033 sucrose · starch. These data were collected at heating times well in excess of that required to destroy all reducing sugars present. Heating times were reduced in the second experiments such that kinetic data could be obtained. The average E_a was 28,500 calories/mole°K and the average reference reaction rate at 100°C (K_{100}) was 0.036 mg lysine/min.

INTRODUCTION

THE POPULARITY of cereal-type snack foods in this country and the fact that people in many undernourished areas of the world subsist on cereal based diets have created interest concerning the nutritive losses these products suffer during thermal processing. A major source of nutritive loss during processing is the deterioration in protein quality caused by nonenzymatic browning (Maillard browning). Maillard browning primarily involves the reaction of free amino groups and reducing sugars (Hodge, 1953). The majority of free amino groups participating in the reaction are the amino groups of lysine (Hannan and Lea, 1952). This is particularly important since lysine is an essential amino acid and it is most often the limiting amino acid in cereal products.

The volume of literature on Maillard browning is too vast to cite completely. The commonly cited reviews by Hodge (1953, 1967), Bender (1972), Braverman (1963), Ellis (1959) and Reynolds (1963, 1965) serve to cover most of the earlier research. The literature concerning lysine loss during food processing is summed up by Carpenter (1973). Our research involved studying lysine loss during processing since several investigations have shown lysine availability to be a reliable indicator of losses in protein quality due to Maillard browning (Lea and Hannan, 1950; Carpenter, 1973). Our choice for monitoring lysine loss was also based on its most often being the limiting amino acid in cereal diets.

The objective of this study was to develop a mathematical model for the prediction of losses in protein nutritive value (as indicated by lysine availability) of any cereal of known composition and processing conditions. It would be then possible to predict the final nutritive value of a cereal protein following thermal processing without conducting tests on each product. Such a model might enable the food manufacturer to manipulate his process to optimize retention of nutritive value where Maillard browning occurs. The work described involves a study of lysine losses during processing of a model food system of varying composition and processing conditions.

MATERIALS & METHODS

Sample preparation

The model system consisted of soybean protein (20%-Promine D, Central Soya), glucose (0–4%), sucrose (0–4%), potato starch (0–10%) and microcrystalline cellulose (Avicel, FMC Corp.) to make 100%.

Desired amounts of the components were weighed and dry blended. The blended samples were slurried with a minimum volume of 0.1M buffer to bring the pH to the desired level (pH 4 and 5.5–phthalate buffer, pH 7–phosphate buffer, pH 8.5–boric acid buffer and pH 10–sodium bicarbonate buffer). Minor pH adjustments were made with acid or base. The pH adjusted slurries were placed in shallow pans, frozen and freeze dried. The dried sample was crushed using mortar and pestle and then about 10g was placed in a plastic weighing boat for a_w adjustment. The samples were equilibrated to the desired a_w in desiccators under vacuum over saturated salt solutions [a_w 0.33–$MgCl_2$, 0.51–$Mg(NO_3)_2$, 0.65–$CuCl_2$, 0.84–KCl and 0.93–KNO_3].

After 2 or 3 days the samples were removed from the desiccators and mixed. Half of each sample (approx 5g) was placed in a foil pouch and vacuum sealed. The pouches were heated at 105 ± 0.1°C or 130 ± 0.1°C in miniature retorts for varying lengths of time. Samples to be heated at 80°C were placed in a water bath. Immediately after heating the pouches were immersed in an ice bath until cooled to room temperature.

Sugar analysis

Glucose and sucrose were determined using a Hewlett-Packard model 7620A Research Gas Chromatograph (GC) equipped with an automatic liquid sampler (Hewlett-Packard model 7670A) and electronic integrator (Hewlett-Packard model 3370B). One ml water, 0.5 ml inositol (1.5% soln) and 0.5g sample were placed in a glass vial, frozen and freeze dried. Two ml TriSil (Pierce Chem. Co., Rockford, Ill.) were added to the dry sample and mixed. After 5 min, a 2 μl sample was injected into the GC. The column was 1.8m × 2 mm i.d. glass packed with 3% SE-30 on 80/100 mesh Chromosorb W-HP. A flame ionization detector was used and the column was programmed from 160–290°C at 20/min with an 8 min hold. Inositol served as an internal standard for quantification purposes.

Available lysine

Available lysine was determined using the fluorodinitrobenzene (FDNB) procedure of Carpenter (1960) with the modifications proposed by Booth (1971). The method entails the reaction of sample with FDNB, acid hydrolysis and colorimetric measurement of the extracted DNP-lysine at 435 nm. A methoxycarbonyl chloride treated blank is prepared for each sample. No correction factor was applied in the calculation of available lysine.

Experimental design

The experimental design was a modified partial orthogonal central composite. For the first experiment, six independent variables were identified and coded as shown in Table 1.

Fractional replicates of a 2^N factorial, assuming four factor and higher order interactions are negligible, was augmented with replicated

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center points and duplicated star (*) points. In this way the effect of each variable could be determined with the same accuracy as if only one had been varied at a time, and the interaction effects between the variables could also be evaluated. Neither center nor star points were considered for the temperature variable because the anticipated application of the Arrhenius equation negated the need to determine higher order effects. Time entered the design as a variable dependent on temperature. This is shown in Table 1. This was also justified on the basis of the assumed reaction kinetics. The purpose of this design was to determine the response surfaces of the activation energy in the Arrhenius equation and a reference reaction rate as a function of the first five variables in Table 1.

The design of the second experiment was similar to the previous design except that only +1 and −1 values were utilized and variables sucrose and starch were eliminated. The results from Exp I indicated a drastic change in reaction kinetics and reaction rate took place early in the heating period. A series of tests with time as the only independent variable indicated that the times shown for Exp II in Table 1 are well within the initial reaction period. Replications of the center point and possible use of star points were planned, but not undertaken because insufficient statistical differences were found to justify attempts to determine higher order effects. Sucrose and starch were the least important independent variables in Exp I so their levels were held at the center value in Exp II.

Statistical analysis

The data from the first experiment were not analyzed in the intended manner. The change in reaction kinetics prior to the analysis and the evidence of possible increases in available lysine with increasing time precluded the application of the Arrhenius equation. The results from each case were reported as fraction remaining and a stepwise regression program (Draper and Smith, 1968) fit this data to a response surface type equation including first order effects, squared terms and first order interactions. Terms were allowed to enter the equation until the F-test value for the entry of any additional term was less than 3.97 (corresponds to a confidence level of approximately 95%).

The data from the second experiment were utilized to estimate an activation energy and reaction rate for each combination of the first three variables. A procedure similar to the one reported by Gondo et al. (1972) was utilized to adjust for the temperature lag of the samples during the process. The actual sample temperature was measured by thermocouples every ½ sec. These data and initial estimates of the activation energy and frequency factor were utilized in a zero order model to predict available lysine losses to be expected in the actual processes. This value was compared with the measured loss and the process time adjusted to reduce the difference between the values. This iterative method was repeated until the differences between predicted losses and measured losses were minimized. The apparent come up time (difference between the process time and the time length assuring an instantaneous rise and fall of temperature) determined by this method for the miniature retorts was 6.5 sec. This correction is included in the final estimations of activation energies and frequency factors.

The estimated activation energy E_i, and reference reaction rate, k_r, for each combination of the first three variables was compared with the values determined for all of the data taken as a single set, E and k_r by hypothesizing $H_o:E_i = E$ and $H_o:k_{ri} = k_r$ for i = 1 to 8 (Nie et al., 1970).

RESULTS & DISCUSSION

THE FDNB METHOD used for determining available lysine (AL) was chosen after reviewing the literature for procedures available and after preliminary trials using the TNBS method of Kakade and Liener (1969) as modified by Warthesen (1971). The TNBS method required less time but good replication of results was not achieved. The values obtained using the TNBS procedure were at times abnormally high. In unheated controls of the model system, values of AL as percent of protein ranged from 7–12%. Literature values for AL in soybean protein are about 6%. Results obtained using the FDNB were similar to reported values so this method was used throughout the study.

Experiment I. Studies involving extended heating times

The times and temperatures used in this part of the study were chosen in order to achieve 50% destruction of AL present in our model system. It was found that rather drastic heat treatments were needed to achieve this goal. After the data were collected and statistically analyzed, it was found that the reaction had proceeded well beyond the point where useful kinetic data could be collected. However, regression equations and response surface diagrams obtained from the analysis of data from the initial part of this study show some interesting effects.

Table 1–Test variables

Variables	Range	Process and composition codes (0) Center	(+1)	(−1)	(+*)	(−*)
a_w	0.33–0.93	0.65	0.84	0.51	0.93	0.33
pH	4–10	7	8.5	5.5	10	4
Glucose	0–4%	2%	3%	1%	4%	0%
Sucrose	0–4%	2%	3%	1%	4%	0%
Starch	0–10%	5%	7.5%	2.5%	10%	0%
Temp (°C)	80–130	105	130	80		
Time (min)						
Exp I	30–1440	90	30	1440		
Exp II	2–300	–	2	300		

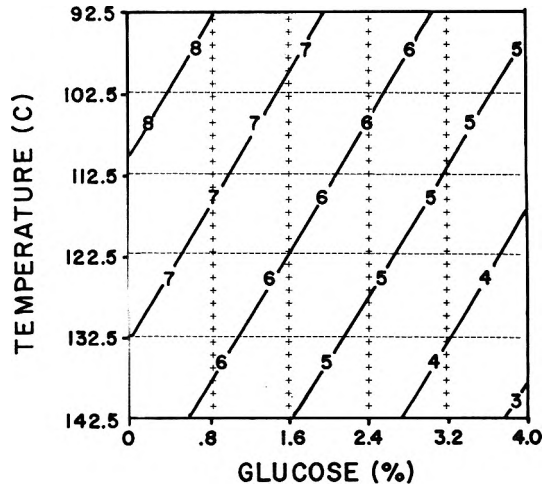

Fig. 1–Effect of temperature and glucose concentration on lysine retention.

At the 95% confidence level: Equation I

F test = 3.97

L = 0.581 + 0.047 pH − 0.093 glucose − 0.059 temperature − 0.0068 time + 0.031 (water activity)^2 + 0.025 water activity • sucrose + 0.033 sucrose • starch

L = fraction of AL remaining

F test of the significance of the equation = 19.6

$R^2 = 0.68$

From the equation it can be seen that the variables most affecting the Maillard reaction and resultant loss of AL were glucose, temperature, water activity squared, the interaction between starch and sucrose and the interaction between water activity and sucrose in decreasing order of significance. Time had a relatively small effect. The R^2 value indicates that these variables explained 68% of the variation in the data.

Response surface diagrams printed out by the computer illustrate the effects of the variables on AL retention. Each diagram shows the effect of two variables as they are varied from −* levels to +* levels. The numbers on the diagram represent fraction of original lysine remaining; for example, 5 represents 50% AL retention. All variables other than the two repre-

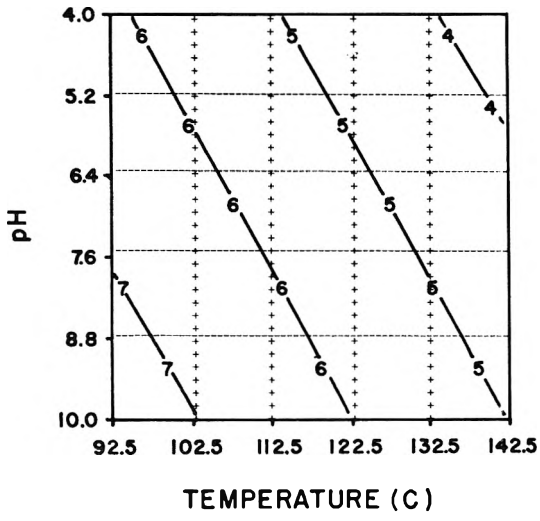

Fig. 2–Effect of pH and temperature on lysine retention.

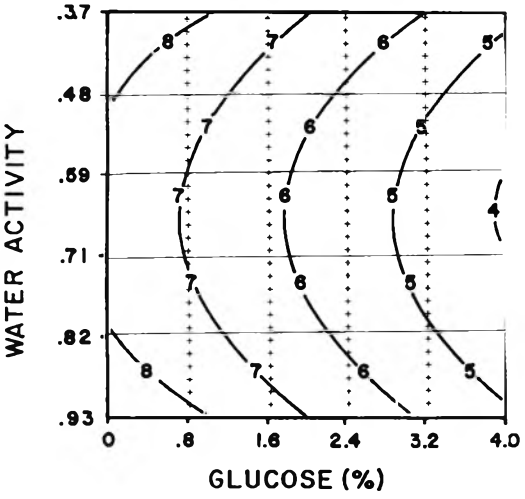

Fig. 3–Effect of water activity and glucose concentration on lysine retention.

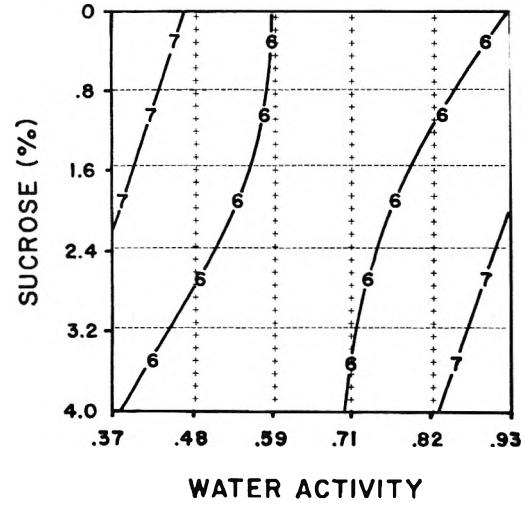

Fig. 4–Effect of sucrose concentration and water activity on lysine retention.

sented on the axes of the graph are held at center (0) levels.

Figure 1 shows the effect of glucose and temperature, the factors shown by equation 1 to have most influence on the destruction of AL. As was expected, retention of AL was greatest at low levels of glucose and low temperatures. The first order terms for glucose and temperature in equation 1 suggest that AL loss with increasing glucose level and increasing temperature is linear. This is shown to be the case in Figure 1.

The effect of pH on the reaction is shown in Figure 2. In this model system AL retention increases as pH increases at any given temperature. This result appears to conflict with results published by other investigators which indicate that the Maillard reaction is accelerated as pH increases. However, in most cases in the literature, the model systems used did not contain sucrose. In the model system used in this research, sucrose was included and glucose was limiting. It is probable that at low pH values and at the time and temperature used, sucrose would be hydrolyzed into the reducing sugars, glucose and fructose. Since the glucose in the model system was consumed early in the heating, the production of additional reducing sugars through sucrose hydrolysis may have resulted in further AL loss. Therefore greater AL loss at lower pH was observed. Further evidence of sucrose hydrolysis was indicated by a pH-sucrose interaction, significant at the 75% confidence level. As pH decreased and sucrose concentration increased, AL underwent greater destruction.

The influence of water activity and glucose on lysine availability is presented in Figure 3. Greater AL loss at higher glucose levels is again shown and at any given glucose level the AL loss is greatest at an a_w of 0.65–0.70. The fact that the effect of water activity is not linear is shown by the squared term for a_w in the equation. This effect of water activity confirms findings of several earlier investigators including Lea and Hannan, (1949) who also worked with a "dry" system and found the browning reaction to be accelerated at 65–70% RH (0.65–0.70 a_w). Browning starts to occur at a water activity of about 0.2. This corresponds to the completion of the bound water portion of the a_w vs moisture content isotherm and the start of the multilayer region of the isotherm. Browning rate increases at increasing a_w until a maximum is reached at an a_w of about 0.65–0.70 where the rate again begins to decrease. The decline in reaction rate is believed to be due to a dilution effect on reactants (Labuza et al., 1970). The possible effect of hysteresis was not studied since all of our samples reached experimental levels of a_w by adsorption.

The interaction between sucrose and starch was shown by equation 1 to have significant effect on the retention of AL in the model system used. This effect may be explained in the same manner as the observed influence of sucrose concentration on the a_w of maximum AL destruction (Fig. 4). The effect of starch and sucrose on AL destruction is believed to be indirect and due to their effect on moisture content rather than a direct participation in nonenzymatic browning. The concept that nonenzymatic browning is related to both a_w and moisture content was suggested by Eichner and Karel (1972). They found that optimum browning rate is determined by the amount of water and state of water binding in a system, and by the mobility of reactants in the system. Maximum browning therefore depends on the extent to which these conflicting influences affect the reaction.

The data presented in Figure 4 show a shift in a_w (0.76 to 0.56) for maximum AL destruction with the addition of sucrose. We believe this is due to sucrose being a good humectant and binding water. At a given a_w, the inclusion of sucrose would require a higher concentration of water in the sample than if no sucrose were present. The higher water content would inhibit the destruction of AL due to both feedback inhibition of the reaction where water is a by-product and through dilution of the reactants. Both moisture content and

a_w appear to have a very significant effect on AL losses in our model systems.

Experiment II. Studies of the kinetics of lysine loss

In this experiment the levels of starch and sucrose were held constant at 5% and 2%, respectively. Only the +1 and −1 levels of the remaining variables were utilized. Shorter heating times (Table 1) were selected for studying the initial rapid reaction that was missed in the first experiment.

The activation energies and the reference reaction rates for each combination of glucose, pH and water activity were determined by least squares analysis based on a zero order reaction. The hypothesis that there was no difference between the activation energy and reference reaction rate for the individual treatments and the respective values for the pooled data was tested statistically. The hypothesis could not be rejected except for the combinations with water activity at 0.34, pH at 5.5 and glucose at either 3% or 1%. The average activation energy, E, was 28,500 calories per mole with a 95% confidence interval of ± 600 calories per mole. This activation energy lies between the values of 26.1 kcal/mol found by Song et al. (1966) and 29.0 kcal/mole reported by Lea and Hannan (1949). The activation energies for the compositions that were significantly different were 19,000 calories per mole for glucose (1%) and 24,600 calories per mole for glucose (3%). The reference reaction rates at 100°C, k_{100} are 0.036 mg/min, 0.028 mg/min, and 0.0054 mg/min for the average results, the treatment with 3% glucose, and the treatment with 1% glucose, respectively.

AL loss was monitored as a function of time at a processing temperature of 130°C and two levels of glucose (Fig. 5). The rates of loss are similar, but AL loss continued longer at the higher glucose level. The linear plot of AL content as a function of heating time indicates a zero order reaction in this model food system. Although the data fits a zero order reaction best, it does not permit us to reject the hypothesis of a first order reaction at the 95% confidence level.

It appears from the graph that lysine availability as measured by Carpenter's method goes through a minimum and then increases slightly with time. This observation was further studied by measuring AL on 60 samples processed for time intervals centering on the processing time for minimum retention. A t-test on the results indicates that the hypothesis that there is an increase can not be rejected at the 98% confidence level.

Figure 5 also shows glucose and sucrose concentration in the samples at pH 8.5 and 1% glucose heated at 130°C for varying times. Glucose is lost in what appears to be a first order reaction, in contrast to the zero order loss of AL. Glucose continues to be destroyed beyond times when AL destruction has ceased probably indicating that glucose is participating in reactions in addition to Maillard browning. One such reaction could be caramelization since the conditions of pH and temperature in these samples would be favorable for this reaction (Greenshields and Macgillivray, 1972). The high pH is not favorable for sucrose hydrolysis and as can be seen from the graph, little or none occurs. Glucose and sucrose concentrations in samples with 1% glucose and pH 5.5 heated at 80°C for varying times were also monitored. Glucose behaved as it did in Figure 5, decreasing past the point where AL loss leveled off. Sucrose, however, differs from Figure 5 in that it decreased slightly as heating time increased, most likely due to hydrolysis which would be favored at the low pH in the samples and the length of heating.

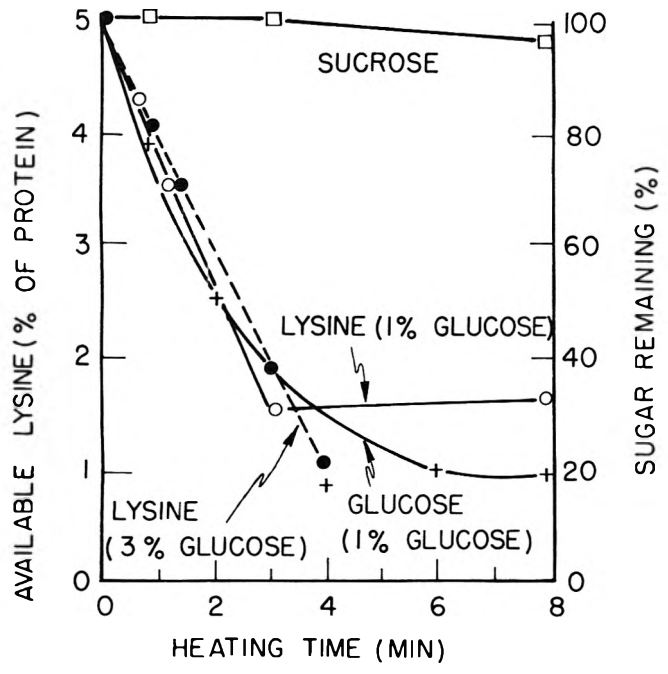

Fig. 5—The effect of heating time on lysine (2 glucose levels) glucose and sucrose destruction (temp 130°C; sucrose 2%; starch 5%; pH 8.5%; and a_w 0.84).

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Ms received 8/22/75; revised 11/13/75; accepted 11/20/75.

Presented at the 35th Annual Meeting of the Institute of Food Technologists, Chicago, Ill., June 8—12, 1975.

Scientific Paper Journal Series No. 9206, Minnesota Agricultural Experiment Station, St. Paul, MN 55108.

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SUPPLEMENTATION OF ONE-POUND LOAVES WITH WET ALKALINE PROCESS WHEAT PROTEIN CONCENTRATES: BAKING AND NUTRITIONAL QUALITY

ABSTRACT

The baking properties, protein quantity and quality of 1-lb wheat flour loaves supplemented with 10–20% wet alkaline process wheat protein concentrate (WAP-WPC) were studied. WAP-WPC was isolated from a pH 9 extract of wheat millrun or shorts by either acid (HCl pH 5) or heat (85°C) and freeze, spray, or drum dried. Acceptable loaves were baked with spray- or drum-dried preparations, with the drum dried, heat precipitated WAP-WPC performing the best. The lysine content of the control bread, 112 mg/g N, was increased from 177 to 206 mg/g N with the incorporation of 10–20% WAP-WPC. Protein digestibility of loaves supplemented with WAP-WPC prepared by various methods was ≥90% and the PER ranged from 1.3–1.7 as compared to 0.76 for the wheat flour control.

INTRODUCTION

IMPROVEMENT of the quantity and quality of dietary protein has long been a major objective of nutritionists. Bread is an appropriate vehicle for protein supplementation since it is consumed by a broad spectrum of the U.S. and other populations. Since lysine is the limiting amino acid in wheat flour, proteins containing adequate lysine are best suited to compliment wheat protein. Flours, concentrates and isolates derived from cottonseed, peanut, sesame, soy, and sunflower as well as dry milled wheat protein concentrate have been incorporated into breads (Ranhotra et al., 1971; Ranhotra and Loewe, 1974; Rooney et al., 1972; Tsen and Hoover, 1973).

A wet alkaline process has been described in which the protein of wheat millfeeds is extracted, isolated and dried by various methods (Saunders et al., 1975). (Millfeed is a general term applicable to any one of the fractions of the kernel remaining after removal of the flour. Millrun is the entire fraction remaining after flour removal. Shorts is the fraction of millfeed remaining after removal of coarse bran, most of the red dog and germ.) The 5,000,000 tons of millfeeds produced annually in the U.S. represent some 800,000 tons of protein which, if isolated, could be a viable protein source for humans and nonruminants. Wet alkaline process wheat protein concentrate (WAP-WPC) prepared in various ways contains from 50–60% protein (N × 5.7) and from 280–325 mg lysine per g nitrogen (Saunders et al., 1975). The Protein Efficiency Ratio (PER) ranged from 1.8–2.0 (casein corrected to 2.5) with the protein being ≥ 90% digestible (Saunders et al., 1974). Storage studies have shown the lipid fraction of WAP-WPC to be most stable when stored at the original moisture content of the concentrate (Betschart et al., 1975a).

The influence of processing upon the baking quality of WAP-WPC incorporated into laboratory pup loaves has been reported (Betschart et al., 1975b). The objectives of this study were to evaluate the functionality, protein quantity and quality of 1-lb loaves containing from 10–20% WAP-WPC.

EXPERIMENTAL

Materials

The wet alkaline process described by Saunders et al. (1975) was used to prepare WAP-WPC from millrun (Con-Agra-Montana, Inc.) and shorts (Millers' National Federation, Chicago, Ill.). Briefly, the protein was extracted at pH 8.6–9.0, isolated by heat (85°C) or acid (HCl pH 5.0), and freeze, spray, or drum dried. A detailed description of the drying conditions has been previously reported (Betschart et al., 1975a). Temperatures attained during freeze, spray and drum drying were 25°C, 232°C (inlet) and 107–110°C (outlet), and 127–132°C, respectively. Freeze- and drum-dried preparations were milled in a Wiley mill to pass through a 20 mesh sieve.

Methods

Baking procedure and evaluation. 1-lb loaves were baked according to the formula and procedure described in Table 1 (footnote a). Acid or heat precipitated WAP-WPC prepared from millrun or shorts was substituted for 10% of the weight of a Hard Red Winter Baker's Patent Wheat Flour (2.0% N). Heat precipitated, drum-dried WAP-WPC was also incorporated at levels of 15 and 20% as replacement for the wheat flour. Laboratory pup loaves baked with 3% shortening (Crisco) had loaf volumes equal or superior to those made with 0.5% sodium stearoyl-2-lactylate; 1-lb loaves were, therefore, made with the conventional shortening formulation. With each experiment 10% defatted soy flour (Baker's Nutrisoy, Archer Daniels Midland Co., Decatur, Ill.) was included as a treatment. Water absorption of the dough and mixing time, i.e., time required to reach desired consistency, were determined. Loaf volumes were measured after baking by the rapeseed displacement method. External and internal characteristics of the baked loaves were evaluated by criteria as outlined in the Bread Score Report Form of the American Institute of Baking (Chicago, Ill.). Data for each treatment are expressed as the mean of three loaves obtained from a single bake test.

Bioevaluation of protein. The PER was determined by a 21-day study with diets containing 10% protein (AOAC, 1975). The diets were fed, ad libitum, to groups of eight, 21-day-old, male weanling (Sprague-Dawley) rats. The rats were randomly divided into groups in which the mean initial weight was 56g. All rats were individually housed in screen bottomed cages with feed consumption and body weights of each rat recorded weekly. Nitrogen digestibility was determined during the third week of the study and corrected for fecal nitrogen of rats fed a nitrogen-free diet. Statistically significant differences between mean PER values were determined by the Duncan's Multiple Range Test (Duncan, 1955).

Chemical analyses. Amino acid analyses were conducted according to the procedure of Kohler and Palter (1967). Official methods of AOAC were used for proximate analyses (AOAC, 1975). Protein values of all breads were expressed as N × 5.7.

RESULTS & DISCUSSION

THE COMPOSITION of unwashed WAP-WPC used in this study generally ranged from 9.2–9.9% nitrogen, 15–19% crude fat, 1.0–1.2% crude fiber and 4.1–4.9% ash (Table 2). Deviations from these values were observed in the crude fat of the spray dried, acid precipitate from shorts, and in the lower ash and higher nitrogen of the washed sample.

Table 1—Properties of 1-lb loaves baked with wet alkaline process wheat protein concentrate[a]

Sample	Protein[b] (N X 5.7) (%)	Dough mixing properties: Absorption (%)	Mixing time (min)	Loaf vol (cc)	Baking properties: Specific loaf vol (cc/g)	Score[c]: Exter.	Inter.	Total
Wheat flour control	14.1	64	8	2923	6.2	28	58	86
10% Soy flour	17.9	65	7	2297	4.7	23.5	52	75.5
10% WAP-WPC[d] from millrun								
Acid precipitate								
Spray dried (washed)	19.4	65	6	2024	4.1	22	42	64
Spray dried	19.1	65	6	2063	4.2	22	43.5	65.5
Heat precipitate								
Spray dried	18.5	65	6	1463	3.0	17	37	54
Drum dried	18.8	65	6	2615	5.5	27	52.5	79.5
10% WAP-WPC[d] from shorts								
Acid precipitate								
Spray dried	19.1	65	6	2062	4.3	21.5	47	68.5
Heat precipitate								
Spray dried	19.0	67	6	1372	2.7	14	34	48
15% WAP-WPC from millrun								
Heat precipitate								
Drum dried	20.7	67	5	2407	5.0	23	48.5	71.5
20% WAP-WPC from millrun								
Heat precipitate								
Drum dried	23.2	69	5	2240	4.6	20	44.5	64.5

[a] Breads baked using a straight dough procedure with the formula (5): HRW patent bread flour or blend, 100; yeast, 2.5; salt, 2.0; sucrose, 4.0; shortening (Crisco), 3.0; potassium bromate, 10 ppm. Procedure included 70 min fermentation time, 25 min intermediate proof, 65 min pan proof, and a bake time of 20 min at 218°C.
[b] Moisture free basis
[c] Bread score report, American Institute of Baking, Chicago, Ill.
[d] Wet alkaline process wheat protein concentrate; samples unwashed unless otherwise indicated

Properties of the dough

Dough mixing properties were altered with the incorporation of WAP-WPC (Table 1). Absorption increased most when higher levels of the drum-dried, heat precipitate were included. Mixing time decreased with the use of 10% WAP-WPC, decreasing further with the inclusion of 15 and 20% WAP-WPC. The dough handling properties were generally fair, with those of the drum-dried WAP-WPC supplemented dough being good at the 10% level and fair at higher levels.

Baking quality

The baking properties of the various WAP-WPC were significantly affected by precipitation method, drying method, and concentration of the WAP-WPC. Neither source of the WAP-WPC, i.e., millrun or shorts, nor washing during the isolation procedure, had an influence upon baking quality (Table 1). The volumes of loaves baked with drum dried, heat precipitated WAP-WPC at 10–20% levels compared favorably with loaves incorporating equivalent quantities of full-fat or defated soy flour (Ranhotra and Loewe, 1974).

With WAP-WPC extracted from both millrun and shorts, the acid precipitate produced higher loaf volumes and total scores than did the heat precipitate. As shown in Figure 1, grain and texture of the loaves prepared from the acid precipitate were also superior to those containing the heat precipitate. Although the same trends were observed when acid and heat precipitated WAP-WPC were incorporated into pup loaves, it was less pronounced. Also, drum drying eliminated the differences in loaf volumes of pup loaves due to precipitation method, since both acid and heat precipitated, drum-dried concentrates performed equally well (Betschart et al., 1975b).

The drum-dried WAP-WPC performed the best in 1-lb loaves and, when included at levels of 15 and 20%, still resulted in loaf volumes superior to those incorporating 10% of any of the spray-dried preparations (Table 1). As anticipated, the use of increasingly higher levels of the drum-dried WAP-WPC produced loaves of lower volumes and somewhat darker color (Fig. 2).

Total scores followed the same trends as those observed with loaf volumes (Table 1). Flavor and color were, generally, more acceptable in loaves with the greatest volumes and became less desirable as volumes decreased.

Table 2—Proximate composition of wet alkaline process wheat protein concentrates prepared from millrun and shorts (Moisture free basis)

Description[a]	Nitrogen (%)	Crude fat (%)	Crude fiber (%)	Ash (%)
Millrun				
Acid precipitate				
Spray dried (washed)	11.0	19.0	1.1	2.3
Spray dried	9.9	16.6	1.0	4.1
Heat precipitate				
Spray dried	9.2	15.1	1.1	4.5
Drum dried	9.8	16.1	1.1	4.4
Shorts				
Acid precipitate				
Spray dried	9.2	11.9	1.0	4.2
Heat precipitate				
Spray dried	9.7	17.1	1.2	4.9

[a] Samples unwashed unless otherwise indicated

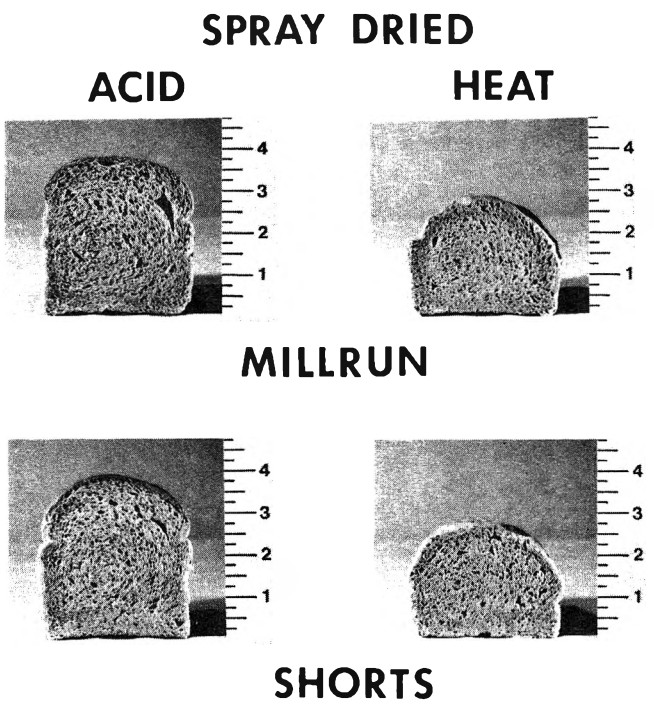

Fig. 1—Cross sections of loaves baked with 10% spray dried, wet alkaline process wheat protein concentrate from millrun or shorts, precipitated by either acid or heat. Numerical scale represents inches.

Since within the spray-dried protein concentrates, those which were precipitated by acid performed best in baking, it appears as though isolation of WAP-WPC by steam injection at 85°C has some deleterious effects upon the functionality of the final product. Higher temperatures encountered during drying, however, seemed to be advantageous. The drum-dried WAP-WPC, which was heated most severely during drying (127–132°C), possessed superior baking properties compared with those which had been spray dried at outlet temperatures of 107–110°C, respectively. It has been reported that heating raw skim milk to 79°C for 10 min, as well as the dry heat treatment of sunflower and cottonseed flour, enhanced baking quality (Swanson and Sanderson, 1967; Rooney et al., 1972). The dry heat treatment of sesame and peanut flour, however, slightly impaired their baking characteristics (Rooney et al., 1972). The influence of heat upon protein preparations prior to being incorporated into breads would seem to be a function of many factors including moisture content, the presence of volume-depressing, heat sensitive substances which may interfere with fermentation and CO_2 production, the composition, configuration and heat sensitivity of the protein, as well as the functionality of the protein in the bread system. This may account for some of the differences observed when various protein preparations have been heated prior to baking.

Protein quantity and quality

Loaves supplemented with 10% WAP-WPC contained from 18.5–19.4% protein which represented an increase of from 31 to 37% over that of the control (Table 1). With levels of 15 and 20% WAP-WPC, the protein content of resultant loaves was 46 and 64% higher, respectively than the control.

Amino acid composition. The essential amino acid content and, thus, the amino acid score of breads supplemented with WAP-WPC, improved progressively as increasing quantities of wheat flour were replaced with WAP-WPC (Table 3). The additional lysine contributed by 15 and 20% WAP-WPC was not, however, proportional to that supplied at the 10% level. The phenomenon of lysine destruction during baking due to the Maillard reaction is well documented. Jansen et al. (1964) reported as high as 30% of the added lysine monohydrochloride was destroyed in breads baked at 232°C for 30 min in the presence of reducing sugar. Losses diminished greatly when baking time was reduced to 20 min. Ranhotra et al. (1971) observed that from 11.7–18.8% of the lysine was lost when breads supplemented with dry milled wheat protein concentrate were baked at 204°C for 25 min. It is apparent that partial destruction of lysine occurs under a variety of baking conditions, including temperatures of 218°C for 20 min which were used in the present study.

Bioevaluation of the protein. One of the major objectives of the wet alkaline process is to enhance the protein digestibility

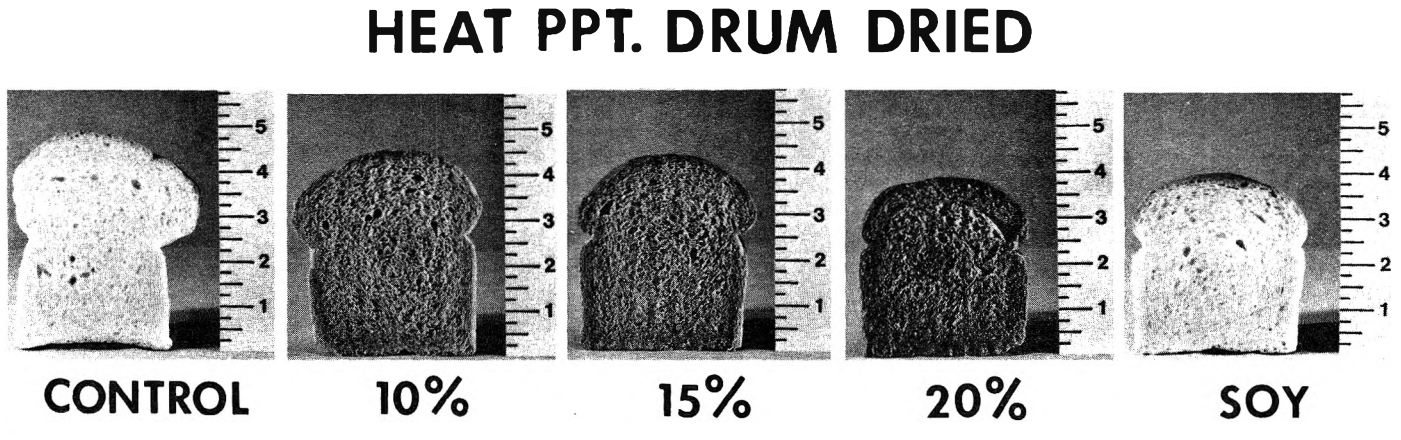

Fig 2—Cross sections of breads baked with wheat flour control, 10, 15 or 20% heat precipitated, drum-dried wet alkaline process wheat protein concentrate from millrun, or 10% soy flour. Numerical scale represents inches.

Table 3–Amino acid composition of select breads containing 10–20% wet alkaline process wheat protein concentrate

Amino acid	FAO provisional pattern[a]	Wheat flour control	WAP-WPC[b] (Heat ppt, drum dried) 10%	15%	20%
	mg amino acid/g nitrogen				
Isoleucine	250	252	256	253	256
Leucine	440	447	456	447	450
Lysine	340	112	177	187	206
Methionine		112	124	123	126
Cystine		160	135	126	128
Methionine + Cystine	220	272	259	249	254
Phenylalanine		304	294	293	289
Tyrosine		206	201	204	198
Phenylalanine + Tyrosine	380	510	495	497	487
Threonine	250	183	207	206	218
Valine	310	259	307	303	329
Histidine		122	143	143	141
Arginine		247	315	331	355
Aspartic acid		264	332	354	383
Glutamic acid		2299	1986	1814	1797
Serine		305	318	299	308
Proline		682	592	525	513
Glycine		219	256	262	275
Alanine		191	242	255	271
Amino acid score[a] (Chemical score)		33	52	55	60.6
% N recovered		95.8	95.7	92.6	92.3
% N in sample		2.48	3.29	3.63	4.07

[a] FAO (1973)
[b] Wet alkaline process wheat protein concentrate

Table 4–Protein efficiency ratio and digestibility of breads containing 10–20% wet alkaline process wheat protein concentrate

Sample	PER[a]	Digestibility Diet[b]	Digestibility Nitrogen[c]
Casein	2.50a	95	100.6
Wheat flour control	0.76e	97	96.4
10% Soy flour	1.73b	93	92.4
10% WAP-WPC from Millrun[d]			
Acid precipitate			
Spray dried, washed	1.52bcd	94	91.6
Spray dried	1.27d	94	92.4
Heat precipitate			
Spray dried	1.37cd	94	93.4
Drum dried	1.71b	94	90.4
10% WAP-WPC from shorts			
Acid precipitate			
Spray dried	1.42cd	94	92.0
Heat precipitate			
Spray dried	1.56bc	94	93.6
15% WAP-WPC from Millrun			
Heat precipitate			
Drum dried	1.57bc	94	90.3
20% WAP-WPC from Millrun			
Heat precipitate			
Drum dried	1.44cd	94	92.7

[a] Duncan's multiple range test; means without a letter in common are significantly different P = 0.05.

[b] $\text{Digestibility of diet} = \frac{\text{Feed intake} - \text{Fecal wt}}{\text{Feed intake}} \times 100.$

[c] $\text{N Digestibility} = \frac{\text{N Intake} - (\text{Fecal N} - \text{Endogenous fecal N})}{\text{N intake}} \times 100.$

[d] Wet alkaline process wheat protein concentrates; samples unwashed unless otherwise indicated.

by separating the protein from the fibrous material. The protein digestibility of wheat millrun and shorts is typically 72 and 77%, respectively, whereas that of WAP-WPC is generally 92–93% (Saunders et al., 1974). As shown in Table 4, the protein digestibility of breads supplemented with WAP-WPC was $\geqslant$ 90%.

The PER of all breads supplemented with WAP-WPC was superior to that of the wheat flour control (Table 4). Breads containing 10% WAP-WPC exhibited PER values from 1.27–1.71 with the mean being 94% greater than that of the control. Although PER values for 21-day studies might be expected to be slightly inflated, Hegarty (1975) reported that the mean of many 20–21-day studies was not more but 13% less than that for 28-day studies. Thus, the values reported in the present study should be somewhat indicative of a 28-day study.

Although there were variations in the PER values of breads supplemented with WAP-WPC, there were no significant differences as a function of source (millrun or shorts), precipitation method (heat or acid), or washing procedure prior to drying (Table 4). Drying method did influence PER with breads supplemented with drum-dried WAP-WPC having a significantly higher PER than those containing the unwashed spray-dried preparations from millrun at the 10% level. Concentration of the WAP-WPC used also had an effect upon PER. Although the PER of loaves supplemented with 10 and 15% of the drum-dried WAP-WPC were not significantly different, the PER of loaves containing 10% WAP-WPC was significantly higher than that of loaves with 20%. The higher concentrations of lysine in the 20% formulations are apparently not completely available to the rat. Although results are not entirely analogous, Ranhotra et al. (1971) reported progressively smaller increases in PER as levels of dry milled WPC in breads were increased. The loss of lysine is an especially critical phenomenon in baked products which are intensively fortified with protein and/or lysine.

CONCLUSIONS

THE BAKING QUALITY of WAP-WPC was influenced by precipitation and drying method, and by level used. Within the spray-dried preparations, the acid precipitates were superior to the heat precipitates. The drum dried WAP-WPC at the 10% level performed best and produced volumes which compared favorably with reported volumes for soy-fortified breads. The color and flavor of WAP-WPC supplemented breads were similar to that of whole wheat bread.

The protein content of breads supplemented with WAP-WPC was increased 31–64% over the control with the replacement of 10 and 20% of the flour, respectively. With the incorporation of 10% WAP-WPC the lysine content of loaves increased 58% over the control, and PER values ranged from 1.27–1.71. Although the lysine content increased with the incorporation of increasing quantities of WAP-WPC, it was not, apparently, as available at the higher levels. Since lysine destruction is a potential problem with many baked goods, further studies are needed to specifically define the influence and interaction of factors such as ingredients, i.e., concentration of

the protein and reducing sugars, baking time, and temperature upon lysine availability.

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Ms received 10/28/75; revised 2/10/76; accepted 2/16/76.

The authors thank T.A. Lehmann for technical assistance in baking, M.E. Allis for amino acid analyses, A.N. Booth for bioassays, R.H. Edwards for pilot plant preparations and H.M. Wright for proximate analyses.

Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Dept. of Agriculture to the exclusion of other that may be suitable.

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EVALUATION OF THE QUALITY, ACCEPTABILITY AND TASTE OF SOY-FORTIFIED BUNS

ABSTRACT

Hot dog buns were prepared from wheat flour and wheat flour fortified with 12% defatted soy flour; 12% soy-fortified buns contained 27.5% more protein and 29.2% more minerals than wheat buns. Soy-fortified buns were slightly larger and darker with grain similar to that of wheat buns. Comparative acceptability and taste of the buns, served with hot dogs during lunch at five elementary schools, were evaluated by about 50 students at each school a day. Soy-fortified buns were rated acceptable by 85% of the children. The taste of the soy-fortified buns did not differ significantly ($P < 0.05$) from that of the wheat bun according to the statistical results.

INTRODUCTION

CEREALS are generally deficient in lysine and some other essential amino acids and, therefore, inferior in protein quality nutritionally. Most cereals are also low in protein. In general, cereals alone will not provide the quality and quantity of protein needed by young children; the nutritional benefits of amino acid and protein fortifications are well documented (Milner, 1969; Scrimshaw and Altschul, 1971).

Of all cereal foods, bread is the most popular staple food in the world. Its high consumption, wide acceptability, and low price make it an ideal food to be fortified for various feeding programs, including school lunch programs, and for general public consumption.

Many protein-rich additives can be used as protein-fortifiers. Defatted soy flour is a favored fortifier for its price, availability, protein content, and quality. However, fortifying wheat flour with soy flour at more than 6% can adversely affect dough properties and bread quality (Tsen, 1971). But the improving action of sodium stearoyl-2 lactylate, calcium stearoyl-2 lactylate, and ethoxylated monoglycerides alleviate the adverse effects. Tsen and Tang (1971) and Tsen and Hoover (1973) developed a blend of wheat flour fortified with 12% soy flour and 0.5% SSL. It was later modified to provide two products: 6% and 12% soy-fortified bread wheat flours. The fortified flours are enriched with vitamin A, thiamine, riboflavin, niacin, calcium and iron. SSL and bromate are included to improve loaf volume and bread quality. The two blends' specifications were developed largely through the work of the USDA Western Regional Research Lab., ARS, with the cooperation of Kansas State University and Industrial Laboratories (ASCS, 1973). More than 35 million lb of such fortified flours were purchased for use in the Food for Peace program in 1973 and more than 100 million lb for 1974–75 (Shaughnessy, 1974). The soy-fortified flours are being used to prepare breads or buns for school lunch programs in about 30 countries. However, little information has been available concerning the quality and acceptability of soy-fortified breads or buns prepared for such uses.

This study examined the quality of buns prepared from soy-fortified flour and assessed their acceptability in school lunch programs.

METHODS

WE USED COMMERCIAL wheat and defatted soy flours. Their moisture, protein, ash and fat contents were:

	Moisture (%)	Protein[a] (%)	Ash (%)	Fat (%)
Wheat flour	11.8	13.8	0.6	–
Soy flour	11.7	50.8	5.7	0.7

[a] Protein content: %N X 5.7 for wheat flour, %N X 6.25 for soy flour

All analyses were by AACC Methods (1970), except that fat was determined by AOCS Method Aa 4-38 (1971) with petroleum ether as the extracting solvent.

The K-State Process (Tsen and Tang, 1971) was modified for making soy-fortified hot dog buns.

The formula, on a wheat flour or soy-fortified flour (88 parts of wheat flour and 12 parts of soy flour) basis, called for 4% yeast, 12% sugar, 2% salt, 2% shortening (hydrogenated vegetable oil), 0.5% SSL, 70 ppm bromate, 58% water for wheat flour and 78% water for soy-fortified flour. Amounts of water used to prepare various solutions and suspensions were included as parts of the total water required in the formula.

Flour or soy-fortified flour was mixed with sugar-, salt- and bromate-solutions, yeast-suspension and SSL-suspension (55°C) in a standard vertical Hobart A-200 mixer at first speed (low) for 30 sec. The mixer was equipped with a MacDuffee type bowl and fork, and a water jacket to regulate dough temperature. Shortening was added and mixed 30 additional sec. The dough then was mixed 5 min at second speed (medium). The mixed dough temperature was 31–32°C. The dough was placed in a glass container and fermented 40 min at 30°C and 85% relative humidity. After it fermented, the dough was moulded, panned, pressed for 13 min, and proofed at 36°C and 92% relative humidity for 60 min. Baking was at 204°C for 12 min.

Loaf weight, expressed in g, and volume, in cc by seed displacement, were measured within 10 min after buns were removed from the oven and averaged from duplicates. The measurements of the buns used in the school evaluation were repeated each day during the testing period. Average data of these measurements are reported in Table 2.

The grain of finished buns was scored from 1–10 after baking. A bun scoring less than 5 was regarded as unsatisfactory. The rating is, however, arbitrary, for there are no standards for grain-scoring in the baking industry.

Crust and crumb colors were evaluated with Agtron multi-chromatic abridged reflectance spectrophotometer Model M-300A with monochromatic spectral lines: red (640 nm), green (546 nm), and yellow (585 nm). The instrument was standardized with standard disc M-68 and 00 to read 100 and 0, respectively. Slices about ½ in. thick from the crust and the middle portion of a bun were used for measurements.

Acceptability and taste of hot dog buns were evaluated at five elementary schools of the same school district in Manhattan, Kansas. Hot dogs were prepared just before lunch. Hot dog contained simply a bun, prepared the previous day and a commercially available, standard weiner, regularly used in the school district. It was then cut into halves. The evaluation was made at one school a day with about 50 Grade 5 and 6 students randomly selected as evaluators, each given two half-hot

Table 1—Protein, ash and fat contents of buns used to test acceptability

Bun	Protein Content (%)	Protein Increase (%)	Ash Content (%)	Ash Increase (%)	Fat (%)
Wheat	14.2		2.4		1.4
Soy-fortified	18.1	27.5	3.1	29.2	1.3

Table 2—Average weights, volumes, specific volumes, colors and grain scores of indicated test buns

Bun	Wt (g)	Vol (cc)	Crust Red	Crust Green	Crust Yellow	Crumb Red	Crumb Green	Crumb Yellow	Grain score
Wheat	43	276	39	16	24	96	86	94	9
Soy-fortified	42	280	30	10	16	89	81	83	9

dogs with buns made from wheat flour and soy-fortified flour, respectively, identified with symbols such as △ and ○, along with other foods served for the school lunch that day. Students were not told what the buns were made of and asked only to evaluate the buns as follows:

EVALUATION OF HOT DOG BUNS

A. _____ The buns are acceptable
_____ The buns are not acceptable
_____ Only △ _____ or ○ _____ is acceptable

B. _____ △ Tastes better
_____ ○ Tastes better
_____ △ Tastes the same as ○

The evaluation was repeated 2-wk later with the same students at the same school. Absentees made fewer students participate in the second evaluation.

RESULTS & DISCUSSION

Increase in protein and mineral contents with soy fortification

Analyses of the buns showed that 12% soy-fortified buns not only contained 27.5% more protein but also 29.2% more minerals than wheat buns (Table 1). The quantitative increases show significant improvement in the fortified buns' nutritive value. In addition, soy fortification can balance the fortified buns' essential amino acids, primarily by supplying lysine from soy flour. As we reported recently, PER values (corrected) obtained in rat feeding tests were 1.3 and 1.9, respectively, for wheat and 12.0% soy-fortified breads in one study (Shamsuddin, 1972), and 1.1 and 2.2 for wheat and 16.6% soy-fortified breads in another study (Tsen et al., 1975). Previously, others also reported improving bread through protein and lysine fortifications (Jansen, 1969; Jansen and Ehle, 1965; Rosenberg et al., 1954; Rosenberg and Rohdenburg, 1952; Hutchinson et al., 1956, 1959).

Soy-fortified buns were slightly larger than wheat buns. However, the fortified buns' crust appeared darker and their crumb was slightly more yellowish than those of wheat buns (Table 2). The browning reaction during baking from the additional protein, amino acids and sugars in soy flour likely explains the darker colors. The color, however, did not affect acceptability of soy-fortified buns, as reported here later. The grain of both bun types was considered excellent.

Acceptability of soy-fortified buns

In statistically evaluating the acceptability of soy-fortified bun, p_s is the proportion of school children who consider the soy-fortified bun acceptable. Let Y = SA/T where SA = number of school children who consider soy-fortified buns acceptable and T = total number of children in the sample.

Table 3—Results of acceptability tests of hot dog buns

School	Eugene Field			Bluemont			Marlatt			Roosevelt			Woodrow Wilson			Totals		Grand
Test no.	1	2	Total	1	2	Total	1	2	Total	1	2	Total	1	2	Total	1	2	total
Buns are acceptable	38	33	71	37	38	75	37	40	77	41	36	77	43	44	87	196	191	387
Buns are not acceptable	2	0	2	1	2	3	0	0	0	1	1	2	0	1	1	4	4	8
Only wheat buns are acceptable	4	7	11	12	7	19	11	5	16	3	7	10	5	3	8	35	29	64
Only soy-fortified buns are acceptable	5	5	10	5	1	6	1	1	2	7	2	9	5	3	8	23	12	35
Total	49	45	94	55	48	103	49	46	95	52	46	98	53	51	104	258	236	494

Table 4—Results of taste tests of hot dog buns

School	Eugene Field			Bluemont			Marlatt			Roosevelt			Woodrow Wilson			Totals		Grand
Test no.	1	2	Total	1	2	Total	1	2	Total	1	2	Total	1	2	Total	1	2	total
Wheat buns taste better	13	23	36	26	33	59	30	23	53	14	19	33	14	13	27	97	111	208
Soy-fortified buns taste better	14	7	21	20	8	28	10	9	19	12	11	23	16	20	36	72	55	127
Wheat buns taste the same as soy-fortified buns	22	16	38	9	7	16	9	14	23	26	16	42	23	18	41	89	71	160
Total	49	46	95	55	48	103	49	46	95	52	46	98	53	51	104	258	237	495

Y is the maximum likelihood estimate of p_s. Data in Table 3 give an estimate of 0.8542 for Y, hence, 0.8542 is the maximum likelihood estimate of p_s. The 95% confidence interval of p_s is (0.8231–0.8853). So soy-fortified buns were rated acceptable by a large majority (82–88%) of the children (at 95% confidence).

Taste of soy-fortified buns

Using data in Table 4, we tested the following hypotheses to see whether soy-fortified buns tasted as good as wheat buns.

Null hypothesis H_o is that the taste of soy-fortified bun is not so good as that of wheat bun; the alternative hypothesis H_A then is that soy-fortified buns tasted as good or better than wheat buns. We used the sign test of Snedecor and Cochran (1973). Let Y = the number of school children who considered the soy-fortified buns as good or better than wheat buns. At $P < 0.05$, we reject H_o when Y is greater or equal to 266. The critical value, 266, is obtained by:

$$k = \tfrac{1}{2}[(495) + (\sqrt{495}(1.65)] \doteq 266$$

Where 495 is the total school children involved in the sample, and 1.65 is the value from standard normal table. In our sample Y = 287. So our statistical data show that the taste of the soy-fortified bun does not differ significantly from that of the wheat bun.

As found in this study, acceptable buns can be prepared from soy-fortified flour. Such fortified buns can be effectively used to improve the nutrition of meals served in school lunch and other feeding programs.

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Ms received 9/23/75; revised 12/4/75; accepted 12/8/75.

Contribution 904, Dept. of Grain Science & Industry, Kansas Agricultural Experiment Station, Kansas State University, Manhattan.

Financial support from USDA, CSRS Regional Research Project NC-132 is gratefully acknowledged. Thanks also are due to Universal Foods Corp. C.J. Patterson, and Archer-Daniels-Midland Co. for supplying yeast, SSL and soy flour.

The cooperation and assistance of Mrs. Sue Greig, Director of Food Service, U.S.D. 383, and her staff are greatly appreciated.

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FLAME STERILIZATION OF SOME TOMATO PRODUCTS AND FRUITS IN 603 X 700 CANS

ABSTRACT

Processing of fruits and vegetables in 603 × 700 cans for institutional use is a common commercial practice. The present time-temperature treatments for assuring commercial sterility are often excessive and unavoidably detrimental to product quality. Using the count reduction system of process lethality evaluation, canned whole peeled tomatoes packed in juice and fruits packed in syrup were experimentally flame sterilized as rapidly in 603 × 700 cans as in 303 × 406 cans.

INTRODUCTION

SINCE THE PROBLEM of acquiring commercial sterilization in acid foods is one of destroying a population of nonpathogenic microorganism which could cause spoilage, processing times and temperature established for tomato products and fruits are not standardized (NCA, 1968a). Canners, with the guidance of NCA and according to their own experience with spoilage, vary the severity of the applied thermal treatment. For example: For whole peeled tomatoes in juice for one can size, the time-temperature treatment may vary from 22 min at 246°F (118.9°C) at 1.6 rpm to 46.5 min at 210°F (98.9°C) at 1.5 rpm; for puree packs of whole tomatoes, the process may be 50 min at 225°F (107.2°C) at 1.9 rpm for 303 × 406 cans, and up to 100 min at 0.6 rpm for 603 × 700 cans. The thermal process for fruits packed in syrup varies similarly and is approximately 1/3 longer for 603 × 700 than for 303 × 406 cans.

With the introduction of Stériflamme (Filper Corp., San Ramon, CA) for acid foods in California, commercial flame sterilization of whole peeled tomatoes in juice in 303 × 406 cans was accomplished in 12 min which included 4 min preheating. In addition, by properly adjusting flame intensities, diet or syrup packed cling peach halves and/or slices, and/or fruit cocktail were simultaneously processed in the same Stériflamme cooker operating from 180–200 cpm per commodity. In another Stériflamme cooker, whole peeled tomatoes packed in puree were processed in 404 × 502 cans in 15 min, with 5 min preheating included, as compared to 26–76 min of agitated retort processing for similar packs.

At present, the largest can size commercially flame sterilized is 404 × 502. In this work, the potentials of Stériflamme processing tomato products and some fruits in 603 × 700 cans are investigated.

EXPERIMENTAL

Commercial agitated retort processes

Diced and whole peeled tomatoes in 603 × 700 cans were inoculated with spores of *B. coagulans* ATCC No. 8038, sealed and commercially processed to determine process lethality. Additional cans were processed without inoculation and used for quality evaluation (Leonard et al., 1975c). The tomatoes, diced and whole, for quality evaluation were lye peeled in a regular cannery operation.

Processes for tomatoes and some fruits were reproduced in a Steritort process simulator (FMC, San Jose, CA). Fill weights, retort temperatures, reel speeds, and times of the commercial operation were used for each commodity. Heat penetration and spore count reduction data were obtained in triplicate.

Stériflamme processes

A laboratory model Stériflamme unit, described by Leonard et al. (1975a), capable of processing one can at a time was used to explore processing parameters for the various fruits and tomato products. Heat penetration data and count reduction data with spores of *B. coagulans* ATCC No. 8038 were obtained in each test. For diced and whole peeled tomatoes in juice, additional cans were processed without inoculation for quality evaluation (Leonard et al., 1975c). These tomatoes were all commercially lye peeled and comparable to packs described in the retort processing section.

For 303 × 406 cans, the laboratory model Stériflamme was equipped with a 1/8-in. wide burner positioned 9/16 in. from the lowest point of the can. For 603 × 700 cans, the burner was 1/4-in. wide and 1/2 in. from the lowest point of the can.

To test the influence of an angled position of the burners, 5% Bentonite solution (56 oz) with 33 rubber stoppers (heatsinks, 54 oz) were used in a 603 × 700 can. The angle position was simulated by burner movement from right to left, with a span of 3½ in./15 sec. At the end of the cycle, the burner returned to original position within 2 sec and a new cycle began. The point of temperature measurement in this experiment was 1 in. from the end on the longitudinal axis of the can.

Process evaluation

In commercial tests with tomato products, process lethality data were obtained only in terms of spore counts as decimal reductions, using the microbiological methods described by Leonard et al. (1975b).

$$\text{decimal reduction} = \log_{10} a - \log_{10} b$$

a = number of spores inoculated *B. coagulans* ATCC No. 8038 (control can)

b = number of spores surviving the heat treatment (processed can)

In the laboratory tests with tomato products where heat penetration data were obtained, process lethality F^{27}_{212} and thermal degradation values C_O were both calculated using the General Method (NCA, 1968b). The value z = 27°F for spores of *B. coagulans* ATCC No. 8038 was experimentally determined in tomato juice. For thermal degradation (C_O), the z value was calculated with $Q_{18°F} = 2$, and the reference $C_O = C^{250.8}_{212} = 1$ min.

In fruit packs, the z value for *B. coagulans* spores ATCC No. 8038 had not been determined, thus lethality was obtained in terms of decimal reduction only. Thermal degradation (C_O) values were calculated with $Q_{18°F} = 2$, as previously described.

Temperature measurement

In-can liquid temperatures were continuously recorded by mounting Ecklund copper-constantan thermocouples (O.F. Ecklund, Cape Coral, Fla.) into either end of the can. The point of measurement, unless otherwise stated, was 3 in. from the end on the logitudinal axis of the 603 × 700 can.

Preparation of samples

Commercially available 603 × 700 beaded cans were used in all phases of this study. The bodies and ends of these cans were 107-lb plate of T-5 (stiff quality) temper.

Cling peach halves and slices. Lye peeled peach halves or slices were packed cold into 603 × 700 plain tin cans with enameled ends, using 72

oz fruit and 35 oz 40° Brix sucrose syrup. The cans were sealed under 10 in. of vacuum. The samples were prepared for heat penetration studies, and inoculated with 5 ml of *B. coagulans* spores ATCC No. 8038 using approximately 1×10^9 spores/ml inoculum.

Fruit cocktail. Commercially packed 603 × 700 cans of sealed but unprocessed fruit cocktail were transported to the processing laboratory. The fruit cocktail was transferred to new cans to obtain heat penetration and decimal reduction data.

Pear halves. Peeled and cored pear halves were packed cold into 603 × 700 plain tin cans with enameled ends using 68 oz pear halves and 39 oz 36° Brix sucrose syrup. Both heat penetration and decimal reduction data were obtained.

Whole peeled tomatoes in juice or puree. Round and var. 13L (pear shaped) tomatoes were commercially lye peeled, hand selected for wholeness, firmness and color and packed into enameled 603 × 700 cans. Tomato fill weight was 95 oz. The round tomatoes were exhausted 9 min at 208° F, covered with 185° F single strength tomato juice and sealed. For heat penetration data, a thermocouple was mounted through the center of the can bottom and the tomatoes were packed around it without being pierced. The cans were inoculated with 5 ml of spores of *B. coagulans* ATCC No. 8038 using approximately 1×10^9 spores/ml inoculum. The inoculum was distributed over the tomatoes. Net closed weight of each can was 107 oz.

Var. 13L tomatoes were packed in puree. The tomatoes were exhausted 13 min at 210° F and covered with 1.06 (13.2° B) tomato puree at 185° F. Cans were inoculated and prepared for heat penetration study as previously described.

Diced tomatoes in juice or puree. Lye or hot water peeled tomatoes were diced into 1/2 in. cubes. Diced tomato fill weight was 93 oz for juice, and 67 oz for puree packs. The cans were exhausted 14 min at 210° F and covered with 185° F single strength tomato juice or 1.07 (15.2° B) puree. The inoculum was mixed in with the diced tomatoes. Net weight of each can was 107 oz. Tomatoes were lye peeled in the cannery operation and hot water peeled in the laboratory.

Evaluation of the double seams

Each can processed was supported on its double seams and rotated up to 80 rpm in the flame sterilizer. The double seams of processed and unprocessed control cans were evaluated using a seam micrometer and a projector (NCA, 1973a).

Double seam integrity

The No. 10 cans were filled with a basal medium to which glucose and ammonium chloride were added (Stanier et al., 1963). Ten cans were heated in the flame sterilizer to 220° F (104.4° C) without rotation, then spray cooled. When the cans ends returned to concave position (∿140° F) while cooling, the double seams were smeared with a suspension of *Aerobacter aerogenes* until the can cooled completely to <90° F (32.2° C). The test was repeated with cans which were rotated 10–80 rpm. The cans were incubated at 86° F (30° C) and observed for swelling.

RESULTS & DISCUSSION

IN A SYSTEMATIC APPROACH, several commercial rotary pressure and atmospheric cooker processes were evaluated using the Steritort process simulator. From the heat penetration data, thermal degradation $C_{21_2}^{59,8}$ and $F_{21_2}^{27}$ values were calculated. Corresponding spore count reductions were simultaneously determined. The data are shown in Table 1.

Using the single can Stériflamme machine, comparable commercial sterility in terms of spore count reduction was attempted in various products. The 603 × 700 cans were preheated to >200° F (93.3° C). Processing parameters (e.g., can rotation, flame intensity and residence times) were modified until heat penetration, as monitored during the process, was steady and similar in character to heat penetration curves experienced in 303 × 406 cans (Leonard et al., 1975a). Flame process schedules which finally achieved equivalent or better than commercial sterility in No. 10 cans are listed in Table 2. For comparison, some commercial data on 303 × 406 cans are also listed. Using flame processing, it was possible to achieve commercial sterility in 603 × 700 cans as rapidly as in 303 × 406 cans.

In Stériflamme processing No. 10 cans, can rotational speeds were considered to be a potential source of problems. The cans were rotated on their double seams, and there was a definite concern for the integrity of the double seams. As rotational speeds were increased, the flame intensity could also be increased, enough to considerably shorten the process time. However, each rpm in natural can rotation for No. 10 cans meant approximately 19 in./min of track (or machine) length, either leading to a gigantic machine or requiring the installation of flat top conveyors at every level to provide the high can rotational speeds within a reasonable space. Communication with Stériflamme machine designers assured us that, if necessary, the cans may be rotated on the body, but natural can rotation should be kept minimal. At first, 22 rpm was recommended as maximum speed, but at the end of the season it was changed to 10 rpm. The results of tests aimed at achieving commercial sterility within the given parameters are shown in Table 3. The variables available (e.g., sample preparation, residence times and gas pressures) were many and the season

Table 1–Evaluation of rotary pressure cooker processes in 603 X 700 (No. 10) cans.

	Processing condition					
Commodity	Time (min)	Retort temp (° F)	Reel speed (rpm)	Minimum $F_{21_2}^{27}$ value (min)	Minimum C_0 value (min)	Minimum decimal red.
Peach halves	30	212	1.70	N.A.[a]	15.9	4.8
Peach slices	18	218	2.55	N.A.[a]	15.4	5.0
Pear halves	30	212	1.50	N.A.[a]	11.0	2.4
Fruit cocktail	18	221	2.55	N.A.[a]	9.0	5.7
Whole peeled tomatoes	28	242	1.70	92.2	42.7	5.7
in juice	22	225	2.00	7.4	12.2	5.2
Diced tomatoes	26	237	1.70	93.9	43.9	5.7
in juice	42	210	0.71	0.7	4.0	2.1
Diced tomatoes						
in puree	53	227	2.35	2.1	6.1	4.2

[a] Data not available

Table 2–Stériflamme processes which accomplished comparative commercial sterility in foods in 303 X 406 and 603 X 700 cans

	Commercial Stériflamme (303 X 406 cans)				Laboratory Stériflamme (603 X 700 cans)			
	Preheat (min)	Riser (min)	Hold (min)	rpm[a]	Preheat (min)	Riser (min)	Hold (min)	rpm[b]
Peach halves	3–4	2–2.5	5–6	45–35	6	4	0	30
Peach slices	3–4	2–2.5	5–6	45–35	6	4	0	30 & 60
Fruit cocktail	3–4	2–2.5	5–6	45–35	6	4	0	30 & 60
Whole peeled tomatoes in juice	4	2	6	38	3	3	3	30 & 80

[a] Longer residence times in sections correspond to slower can rotation.
[b] The higher rotational speed was applied in the riser and 1/3 of cooling.

was short. Although the data listed in Table 3 indicate the feasibility of flame sterilizing foods in No. 10 cans, they are only starting points in optimizing the processes.

Several possibilities of improving flame processing of 603 X 700 cans were tested. Some variables, e.g., vacuum and headspace, that are taken lightly in the canning of acid foods, showed considerable influence.

Vacuum

In flame sterilization of canned foods, the achievement of high vacuum is very important to prevent permanent distortion (buckling) of can ends during thermal processing (Boyd and Bock, 1952; NCA, 1968a, c, 1973b). In retort processing, buckling is controlled when counter pressure is imposed on cans, counteracting the internal pressure developed during heating, whereas in flame sterilization, the can becomes its own pressure vessel without any imposed counter pressure, and the strength of the can ends and the amount of air allowed to remain in a can become critical factors. A definite advantage of higher vacuum in flame sterilization is that it permits processing at higher temperatures for shorter time. In 603 X 700 cans with 5 in. of vacuum, less than 220°F could be reached in the covering liquid before buckling. By increasing the vacuum to 10 in., the processing temperature could be raised to 230–236°F.

To achieve adequate vacuum in 603 X 700 cans for our experiments, exhausting of the particulates, hot filling of the covering medium and steam flow closing were satisfactory, using cans from concurrent commercial operation.

Headspace

The headspace achieved in this work was small but adequate and not necessarily optimum since it was predetermined by the commercial filling weights used. Although the cans provide additional headspace as the ends expand in response to the internal pressure developed during heating, the optimum headspace needs to be established. Over-filling hindered uniform heat transfer and distribution in the product and caused buckling. Slack filling with high vacuum facilitated better heat distribution, but either extreme is not a good manufacturing practice. The USDA (1946) grade requirements limit the maximum gross headspace allowed while NCA (1968c, 1973b) emphasizes the importance of having headspace which allows for product expansion and movement in agitating (retort) processes.

Burner size and position

In preliminary tests (Table 2), before the economics of can rotational speeds were considered, 603 X 700 cans of tomatoes were flame sterilized in 9 min using the 1/4-in. wide

Table 3—Evaluation of experimental Stériflamme processes in 603 X 700 (No. 10) cans

Processing conditions					Minimum F_{212}^{27} value min	Minimum C_0 value min	Min decimal reduction	Repl.
Preheat min @ rpm	Rise min @ rpm	Gas pres. psig	Hold min @ rpm	Cool min @ rpm				
Peach halves								
6 @ 22	4 @ 60	1	15 @ 22	15 @ 22	N.A.[a]	13.9	4.7	4
6 @ 22	4 @ 60	2	8 @ 22	15 @ 22	N.A.[a]	18.1	total kill	1
Peach slices								
6 @ 22	4 @ 60	1	8 @ 22	10 @ 22	N.A.[a]	18.4	total kill	1
6 @ 22	4 @ 60	1.5	4 @ 22	10 @ 22	N.A.[a]	14.4	4.9	1
Pear halves								
4 @ 10	4 @ 10	1.5	4 @ 10	15 @ 10	N.A.[a]	5.0	2.0	3
Fruit cocktail								
6 @ 22	4 @ 60	1.75	none	15 @ 22	N.A.[a]	5.7	3.7	1
6 @ 22	4 @ 60	1.75	4 @ 22	15 @ 22	N.A.[a]	6.4	total kill	1
Whole peeled tomatoes in juice								
4 @ 10	4 @ 60	1.5	6 @ 10	15 @ 10	11.8	12.4	4.6	2
4 @ 10	4 @ 10	1.5	15 @ 10	15 @ 10	13.5	15.8	4.8	3
6 @ 22	4 @ 60	1.5	4 @ 22	15 @ 22	11.7	11.9	4.6	3
Whole peeled tomatoes in puree								
3 @ 22	3 @ 60	1.5	3 @ 22	15 @ 22	12.9	11.2	3.8	2
4 @ 10	4 @ 60	1.5	6 @ 10	15 @ 10	43.0	22.8	3.0	2
4 @ 10	4 @ 10	2	6 @ 10	15 @ 10	22.4	14.9	total kill	1
Sliced tomatoes (stewed) in juice								
6 @ 22	4 @ 60	1	4 @ 22	15 @ 22	5.4	8.3	4.9	2
Diced tomatoes in juice								
3 @ 22	3 @ 60	1.5	3 @ 22	15 @ 22	14.2	12.2	5.8	3
6 @ 22	4 @ 60	1	4 @ 22	15 @ 22	5.9	9.6	5.5	5
4 @ 10	4 @ 10	1.5	15 @ 10	15 @ 10	1.1	4.9	1.6	3
Diced tomatoes in puree								
4 @ 10	4 @ 80	1.5	6 @ 10	15 @ 10	6.7	10.5	5.2	3
4 @ 10	4 @ 60	1.5	6 @ 10	15 @ 10	N.A.[a]	N.A.[a]	2.9	3

[a] Data not available

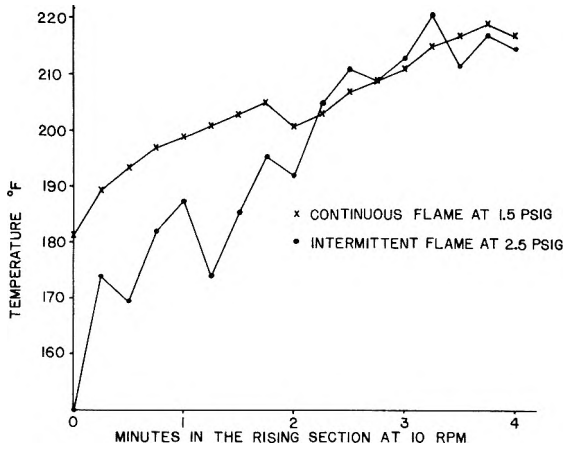

Fig. 1—Comparison of heat penetration data obtained in continuous and intermittent flame heating of canned whole peeled tomatoes in juice in 603 X 700 cans.

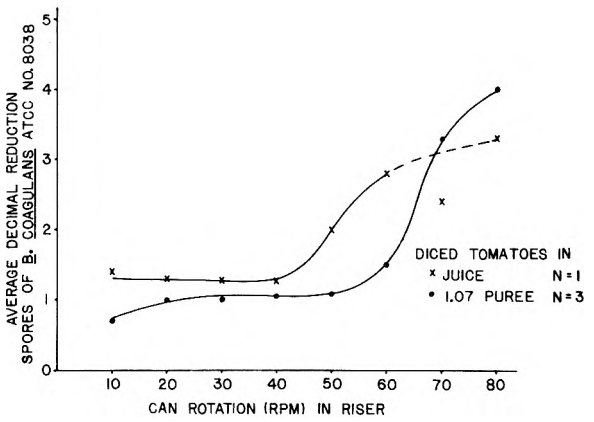

Fig. 2—Influence of can rotation during rising on the lethality of a flame process for diced tomatoes packed in puree and juice, in 603 X 700 cans.

burner and a combination of 30 rpm in preheating, holding and cooling, with 80 rpm in the rising section of the process. This was more rapid than the 12 min commercial process for 303 X 406 cans, using the 1/8-in. burner at constant 38 rpm. Wider burners for larger cans were advantageous, however, using two 1/8 in. wide burners spaced 2–3 in. apart appears more promising, and should be tested when the equipment becomes available.

As an improvement to centered continuous burner position, the effect of burners positioned at an angle was evaluated. The rate of temperature increase averaged 33.4°F/min with the centered burner position and only 31.4°F/min with the simulated angle burner position (significantly lower on the 1% level). The mechanism of heat transfer in Stériflamme needs to be thoroughly investigated.

As an alternative to the application of high speed can rotation, intermittent heating was tested. The time of process was constant except in the rising section. The cans were heated at 10 rpm, using 1.5 psig gas pressure for 4 min to simulate the continuous burner pattern, and 2.5 psig for 30 sec with 15 sec breaks through 4 min 15 sec, to simulate an intermittent burner pattern. The cans of diced tomato packed in either juice or puree buckled at 10 rpm when processed by intermittent heating at 2.5 psig. No increase in bacterial spore reduction or response on the heat penetration curve, as compared to continuous heating, were observed. When the experiment was repeated with whole peeled tomatoes in juice, the cans did not buckle and a good response on the heat penetration curve was observed (Fig. 1). Again, the intermittent burner pattern showed no advantage over the continuous in terms of either processing temperature achieved or the reduction of bacterial spores.

Can rotation

Processing whole peeled tomatoes in juice or puree, and fruit halves or slices in syrup in 603 X 700 cans was effective at minimum 10 rpm, given good vacuum and adequate holding time (Table 3). However, processing of diced tomatoes, whether in juice or in puree, was not adequate at 10 rpm unless high speed rotation was applied in the rising section. The responses of diced tomatoes in juice and in puree to rotational speed in the rising section are presented in Figure 2. Each can was preheated 4 min at 10 rpm, had 4 min on the riser at the indicated rpm using 2 psig gas pressure, and 6 min holding at 10 rpm over minimum flame. Buckling was not a problem at lower rpm's with the gas pressure used, but over-all underprocessing, as indicated by decimal reduction, was evident.

Influence of can rotation on the double seam

The factory ends of the cans used in gaining data for Figure

Table 4—Quality of whole peeled and diced tomatoes packed in juice in 603 X 700 cans, given the indicated thermal processes

	Stériflamme	Stériflamme	Rotary pressure cooker
Whole peeled tomatoes			
Process	9 min	14 min	22 min @ 225° F
Process lethality (decimal reductions)	3.4	4.6	3.5
Drained wt (oz)	86.99	86.41	81.91
1% signif. level[a]	b	b	a
Shear press firmness tests (in. lb X K)	1.75	1.81	1.08
1% signif. level[a]	b	b	a
Agtron E5M color (green/red)[b]	30.3	31.4	32.7
1% signif. level[a]	a	a,b	b
Diced tomatoes			
Process	9 min	14 min	42 min @ 210° F
Process lethality (decimal reductions)	5.8	5.5	2.1
Drained wt (oz)	74.24	71.01	65.38
1% signif. level[a]	b	b	a
Shear press firmness test (in. lb X K)	3.22	3.23	2.81
Not significantly different (10%)	—	—	—
Agtron E5M color (green/red)[b]	31.5	31.7	33.4
1% signif. level[a]	a	a	b

[a] Letters in common do not differ significantly at the level indicated.
[b] Lower values indicate better color.

2 were torn down and examined. Rotational speed did not seem to adversely influence the dimensions of the double seams. The percent overlap averaged higher in the flame processed cans than in the controls. The difference, however, was not statistically significant.

The cans which had the double seams smeared with a suspension of *Aerobacter aerogenes* showed no evidence of double seam failure either. The cans tested showed no evidence of contamination in over 6 months. The small sample size however does not permit a definite conclusion.

Quality of flame sterilized whole and diced tomatoes in juice

The qualities of flame and retort processed tomato samples were evaluated in terms of drained weights, firmness and color (Leonard et al., 1975c). The data are presented in Table 4. The averages are on evaluations of six cans for each commodity and process. Process lethalities shown were single decimal reduction determinations, representing the corresponding time-temperature relationships.

For whole peeled tomatoes, the increased flame process did not significantly influence quality although the lethality was increased considerably. However, the quality of flame sterilized when compared to retort processed tomatoes, was significantly better for the attributes measured. For diced tomatoes the results show similar trends in which the quality of flame processed samples was better and higher lethality levels were achieved. The data confirm the earlier findings of Leonard et al. (1975c).

CONCLUSIONS

THIS RESEARCH indicated that fruits and various canned tomato products in 603 X 700 cans may be flame sterilized as rapidly as the same product in 303 X 406 cans. The quality of the flame-processed foods can be expected to be better than their retort-processed counterparts when the processing parameters are such that the food would be processed at a higher temperature for shorter time. Good manufacturing practices are advantageous for flame processing 603 X 700 cans. The influence of can rotation on the double seams needs to be confirmed with larger samples.

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Ms received 9/16/75; revised 12/9/75; accepted 12/16/75.

The authors thank the Filper Corporation, a DiGiorgio Company, for grants-in-aid to partially support the project.

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TRANSIENT STATE HEAT TRANSFER IN STACKS OF HEAT PROCESSED FOOD STORED IN A COMMERCIAL WAREHOUSE

ABSTRACT

Temperatures in selected locations in the stacks of canned tomato juice and jarred relish, which were placed in an uninsulated warehouse shortly after their production, were determined experimentally. Through careful examination of temperature data collected, mathematical formulas were derived for predicting the temperature response of heat processed food in a warehouse stack. For this derivation, a formula for transient state heat conduction in an infinite slab was used. Since the apparent Biot number and thermal diffusivity values of a warehouse stack were required for predicting product temperature by using the derived formulas, a method was developed to determine these two parametric values. A set of computer programs (Fortran IV) were prepared for the computerized prediction of product temperatures by using the derived formulas because they are too complex for manual calculations. There is reasonable agreement between theoretically predicted and experimentally determined temperatures. The maximum difference between the two is 10F° or less during the initial period of warehouse storage. The computational procedure developed in the present investigation will provide invaluable means for determining the proper warehouse storage of heat processed food.

INTRODUCTION

HEAT PROCESSED FOOD is one of the most stable food products currently available in the market. However, according to results presented by several investigators (Ball et al., 1963; Brenner et al., 1948; Cecil and Woodroof, 1963; Dalal, 1964; Luh and Sioud, 1966; Moschette et al., 1947; Timbers, 1971; Vandercook, 1971; Westcott et al., 1955), the organoleptic and nutritional qualities of heat processed food deteriorated at accelerated rates when it was stored at high temperatures. It has also been observed that storing the food at or about 100°F is highly detrimental to its quality.

The mean temperatures of the food are usually higher than 100°F at the completion of heat processing. This relatively high temperature is required for evaporating residual water droplets on the surface of containers. In many industrial operations, products are placed in carton or other boxes immediately after production and stored in warehouses before distributions through marketing channels. The residual heat of the food should be removed as soon as possible during warehouse storage to eliminate any appreciable loss in quality.

In so far as published literature is concerned, only a few research workers have investigated temperature histories of canned foods during storage in commercial warehouses (Monroe et al., 1949; Porter, 1956; Reister et al., 1948). Monroe et al. (1949) determined air-temperatures during various storage in warehouses as well as the temperatures of canned food in a small six-can lot. Based on the analysis of temperature data collected, they concluded that the temperatures of canned food in a storage warehouse could be estimated from the maximum and minimum air temperatures. This is likely a good estimation of food temperature when products are stored for a long time and when heat transfer in the products equilibrated with surrounding atmosphere. However, their method is not applicable for predicting transient state food temperatures during relatively short periods of warehouse storage since food temperatures are most likely not within the maximum and minimum air temperatures when the food is brought into the warehouse. Porter (1956) investigated changes in canned food and air temperatures in standing railroad boxcars during the summer at Yuma, Arizona. Although this investigation is not on food temperature during commercial warehouse storage, results obtained might be applicable to some of the warehouse storage. There were considerable differences between the maximum air temperature and maximum food temperatures, and it took as long as 2 months for the temperature of canned food samples to reach equilibrium with ambient temperatures. Reister and his coworkers (1948) investigated the warehousing of canned citrus products. The product was piled four pallet-loads high, with a 6-in. space between tiers. They found that mean air temperatures were dependent upon vertical location in the warehouse: the closer to the roof of the warehouse, the higher the mean air temperature.

These published data may provide useful information on food temperature during warehouse storage, but are not readily applicable for predicting food temperature in the initial phase of the storage. In the present investigation, a procedure is developed for this prediction through the careful examination of temperature data collected.

TRANSIENT STATE FOOD TEMPERATURE EXPERIMENTALLY DETERMINED

Text procedure

The temperature history curves of heat processed food were determined during storage in a commercial warehouse. These curves determined are utilized for developing a procedure for predicting the transient temperatures of food stored in a warehouse.

Products used for the present investigation are 303 × 406 cans of tomato juice and 2 oz. jars of relish. The food was processed in a commercial cannery in New Jersey on summer days of 1968. Immediately after processing, 24 cans or jars of finished product were filled in a carton. Cased products were then brought to a nearby commercial warehouse for initiating storage tests.

The warehouse was of a common storage type, an uninsulated metal building not equipped with any special ventilation or temperature control features, except a heating system is used in winter months.

Temperatures were monitored at several locations in warehouse stacks using a potentiometric recorder and copper-constantan thermocouples. The typical locations of the thermocouples in a stack are shown in Figure 1.

The configuration of the stacks used in this particular warehouse was the type known as a "pull-pallet" or "slip-sheet." No conventional wooden pallets were used except between the floor and the first layer of cases. Pallet loads were separated by a single sheet of cardboard and, consequently, there was no circulation of air between the layers of the stack. A 2–4 in. space was left between the rows of stacks to provide

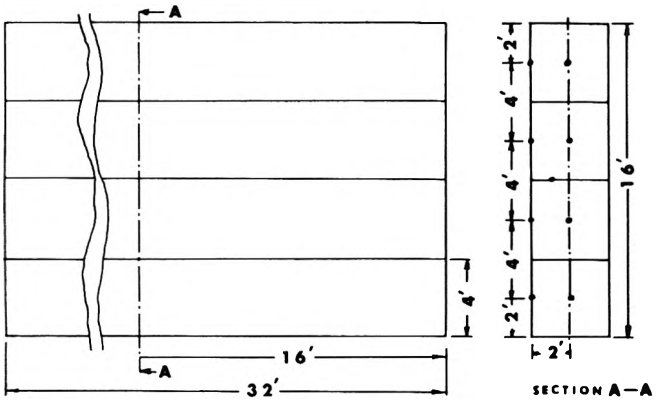


Fig. 1—Location of thermocouple junctions placed in a warehouse stack of heat processed food.

air circulation, resulting in a stack configuration about 4 ft thick, 30 ft long and 16 ft high.

Thermocouples were placed in pallet loads as they arrived at the warehouse from the processing and packing facilities just prior to being placed in the stack. Following completion of the stack, all thermocouples were connected to the recorder.

A complete set of data was obtained from the test for canned tomato juice. However, because of electrical failure, no data were collected between 48 and 136 hr of storage of the jarred relish.

Experimental temperature history curves

Results, which were obtained from the test with canned tomato juice, are presented below. The temperatures of the pull-pallet stack of cases as well as surrounding air temperatures are shown in Figure 2. Even though data were collected for more than 1 month, those collected during the latter part of the storage test are not given in this figure because of the relatively slow rate of change in the product temperatures. The surface temperature of the stack fell rapidly toward the air temperature and oscillated within the range of the air temperature after four diurnal cycles. Center temperatures fell quite slowly, with a period in excess of a month required for it to approach the mean of the ambient air. Virtually no diurnal temperature fluctuation was observed at the center of the stack and even at $x/\ell = 0.5$, half-way between the surface and mid-plane, the diurnal temperature variations were damped out.

The air temperature within the warehouse shows fair variation depending upon location as pointed out by Reister et al. (1948). The amplitude of the temperature oscillation is much greater near the top of the stack than near the bottom. The mean air temperature is somewhat higher nearer the ceiling. However, the vertical temperature gradient in surrounding ambient air has little influence on the center temperatures at the four levels observed, Figure 3. Differences in temperature at the stack mid-plane and at levels between 2 and 14 ft above the floor are small, with the maximum difference in the order of 5 F°. The two intermediate levels at about 6 ft and 10 ft above the floor had the slowest cooling rate. For the first 12 days of storage, the centers of the top and bottom pallets were very similar. After 12 days, the temperature of the top pallet decreased more slowly and approached the temperature of the two intermediate levels. By the end of the test period (40 days), the temperature of the top pallet was the warmest, about 1 F° in excess of the mid-level and 3 F° above that of the low level.

Results obtained with jarred relish are similar to those described above. Some results with this product are given later under "Estimation of stack temperature."

DEVELOPING A PROCEDURE FOR PREDICTING TRANSIENT STATE FOOD TEMPERATURE DURING WAREHOUSE STORAGE

THERE ARE complicated physical processes involved in heat transfer from a stack of heat-processed food to the surrounding atmosphere in a warehouse. Heat is likely transmitted from food in metal containers to the atmosphere through the combination of conduction, convection and radiation. A mathematical model for the heat transfer, based on these three transport processes, is highly complicated and it is extremely difficult to derive usable solutions for estimating food temperatures for this model. Even if we obtain solutions, they have

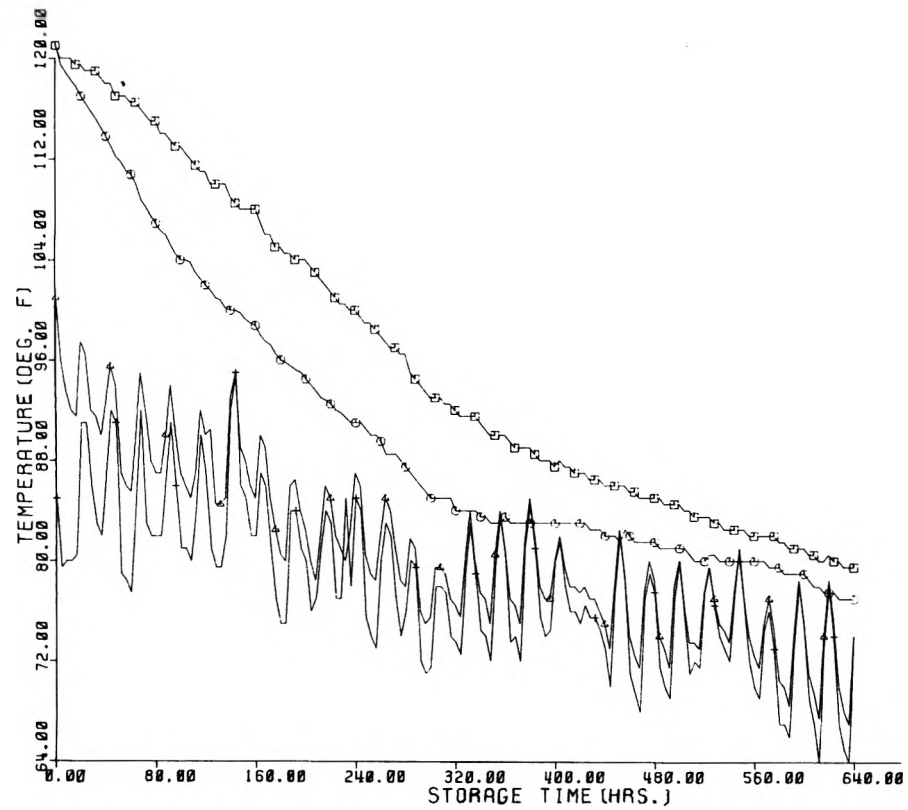


Fig. 2—Experimentally measured temperatures at selected locations in mid-height level of a warehouse stack of canned tomato juice.

center
midway
surface
air

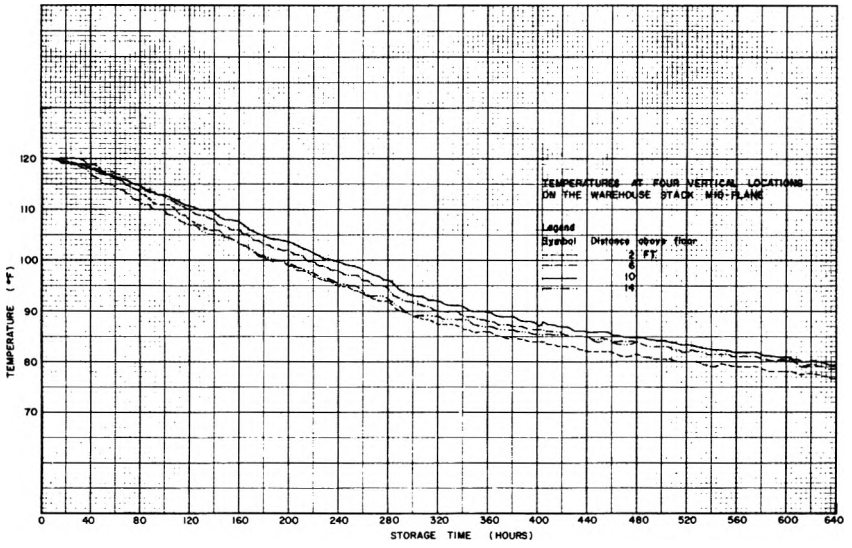


Fig. 3–Experimentally measured temperatures at vertical locations on a warehouse stack of canned tomato juice.

greatly limited practical use because of the computational efforts required.

In order to develop a usable and reliable formula for estimating food temperature, we carefully examined the temperature history curves of warehouse stacks, which were obtained in the present investigation. The slow cooling rate at the center of the stack and the marked damping of the daily temperature fluctuations indicate that heat conduction is a major mechanism for heat transfer in the stack. It is also observed that the stack may be assumed to be approximately homogeneous, even though it is a composite of food, containers, void spaces and cartons, and that the effective thermal diffusivity value of the stack is not large and/or the surface film conductance is low.

A body of brick shape may be considered as that of the cross-sectional volumetric element which is formed when three infinite slabs perpendicularly intersect each other. In previous investigations (Hayakawa, 1974), we observed that heat conduction in the body of brick shape could be approximated with that in an infinite slab if the following conditions are met.

(1) This infinite slab is identical to one of the three intersecting slabs, whose thickness is the smallest among them.
(2) This smallest thickness is at most one-half of those of the other two slabs.
(3) Biot number applicable to the body of a brick shape is greater than or equal to 2 when the half length of its shortest edge is used as a characteristic dimension for estimating this number.

Heat transfer in warehouse stacks is approximated by the following one-dimensional heat conduction equation because of the above stated observations.

Heat conduction equation:

$$\frac{\partial \theta}{\partial t} = \alpha \frac{\partial^2 \theta}{\partial x^2} \tag{1.1}$$

Boundary condition:

$$\frac{\partial \theta}{\partial x} = 0 \qquad x = 0 \qquad \text{and} \qquad t > 0 \tag{1.2}$$

$$\frac{\partial \theta}{\partial x} = H \left\{ V \sin(\omega t + \epsilon) + Rt + p - \theta \right\}_{x = \ell \text{ and } t > 0} \tag{1.3}$$

Initial condition:

$$\theta = 0 \qquad t = 0 \qquad \text{and} \qquad 0 < x < \ell \tag{1.4}$$

Eq. 1.2 is applicable to the central plane of the stack and indicates that temperature distribution in the stack is symmetrical with respect to this plane. According to our observation, ambient temperatures in the warehouse did not only oscillate diurnally but also the center of this oscillation changed linearly with time. Therefore, Eq. 1.3 is assumed to approximate the boundary condition applicable to the exposed surface of the stack. In this equation, the following expression is used to represent the changes in the ambient temperatures:

$$\theta_{am} = V \sin(\omega t + \epsilon) + Rt + p \tag{1.5}$$

According to our observation, the initial temperature distribution in the stack was almost uniform, therefore, Eq. 1.4 is assumed. It should be noted that all temperature variables in the above equations are obtained by subtracting the initial temperature of the stack from thermometric temperature. Eq. 1.1 was solved analytically by applying Laplace transformation. The solution obtained is given below.

$$0 = V\theta_{ss} + V\theta_{st} + \left(\frac{R\ell^2}{\alpha}\right)\theta_R + P\theta_p \tag{2.1}$$

where

$$\theta_{ss} = \frac{BiM}{N} \sin(Fo/Fm + \epsilon + \phi - \eta) \tag{2.2}$$

$$\theta_{st} = 2\,Bi \sum_{j=1}^{\infty} \frac{\beta_j \left(1/Fm\right) \cos\epsilon - \beta_j^2 \sin\epsilon \cdot \cos(\beta_j \rho)}{(\beta_j^4 + 1/Fm^2)(\beta_j^2 + Bi^2 + Bi) \cdot \cos\beta_j} \cdot \exp(-\beta_j^2 Fo) \tag{2.3}$$

$$\theta_R = Fo + (1/2)(\rho^2 - 1 - 2/Bi) + 2Bi \sum_{j=1}^{\infty} \frac{\cos(\beta_j \rho)}{\beta_j^2 (Bi^2 + Bi + \beta_j^2) \cos\beta_j} \cdot \exp(-\beta_j^2 Fo) \tag{2.4}$$

$$\theta_p = 1 - 2Bi \sum_{j=1}^{\infty} \frac{\cos(\beta_j \rho) \sec\beta_j}{Bi^2 + Bi + \beta_j^2} \cdot \exp(-\beta_j^2 Fo) \tag{2.5}$$

and where

$$Bi = h\ell/k \tag{2.6}$$
$$Fo = \alpha t/\ell^2 \tag{2.7}$$
$$Fm = \alpha/(\ell^2 \omega) \tag{2.8}$$

$$M = [\{\cosh(\xi\rho)\cos(\xi\rho)\}^2 + \{\sinh(\xi\rho)\sin(\xi\rho)\}^2]^{1/2} \quad (2.9)$$

$$N = [\{\xi\sinh\xi\cos\xi + Bi\cosh\xi\cos\xi - \xi\cosh\xi\sin\xi\}^2 + \{\xi\sinh\xi\cos\xi + Bi\sinh\xi\sin\xi + \xi\cosh\xi\sin\xi\}^2]^{1/2} \quad (2.10)$$

$$\xi = \ell\sqrt{\omega/(2\alpha)} = \sqrt{1/(2\,Fm)} \quad (2.11)$$

$$\rho = x/\ell \quad (2.12)$$

$$\phi = \arctan[\{\sinh(\xi\rho)\sin(\xi\rho)\}/\{\cosh(\xi\rho)\cos(\xi\rho)\}] \quad (2.13)$$

$$\eta = \arctan[\{\xi\sinh\xi\cos\xi + Bi\sinh\xi\sin\xi + \xi\cosh\xi\sin\xi\}/\{\xi\sinh\xi\cos\xi + Bi\cosh\xi\cos\xi - \xi\cos\xi\sin\xi\}] \quad (2.14)$$

In Eq. 2.1, θ_{ss} and θ_{st}, respectively, represent quasisteady state temperature distributions and transient state temperature distributions in a slab, which is exposed to this ambient temperatures: $\sin(\omega t + \epsilon)$. The expression in the third term, θ_R, is related to the temperature response of a slab when it is exposed to linearly changing ambient temperatures. The expression in the last term, θ_p, is transient state temperature distributions in a slab when there is a step change in ambient temperatures. Formulae, which are identical to Eq. 2.1 through 2.5 may be obtained by applying Duhamel's theorem to a proper temperature response function (Carslaw and Jaeger, 1959).

Since the warehouse is uninsulated, air temperature at internal storage space is directly influenced by changes in weather conditions. Because of this influence, the amplitude of sinusoidal variation, V, and the rate of linear change, R, in air temperature likely change during storage. In addition to this, there may be sudden changes in air temperature when warehouse doors are opened for a relatively long time or when trapped hot air in the warehouse is exhausted by using a ventilation fan. Therefore, another formula is derived by assuming the following surrounding air temperatures.

$$\theta_{am} = \begin{cases} V_1\sin(\omega t + \epsilon_1) + R_1 t + p_1 \\ V_m\sin(\omega t + \epsilon_1) + R_m(t - t_m) \end{cases} \quad t_1\,(=0) < t \leqslant t_2$$
$$+ \sum_{j=1}^{m=1} R_j(t_{j+1} - t_j) + \sum_{j=1}^{m} p_j \quad (3.1)$$

$$m = 2, 3, \ldots, n \quad \text{and} \quad t_m < t < t_{m+1} \quad (3.2)$$

A formula, which is applicable to the above air temperature, is:

$$\theta = \sum_{i=1}^{m}[(V_i - V_{i-1})\{\theta_{ss}(Fo - Fo_i)_{\epsilon_i = Fo_i/Fm + \epsilon_1} + \theta_{st}(Fo - Fo_i)_{\epsilon_i = Fo_i/Fm + \epsilon_1}\} + \frac{\ell^2}{\alpha}(R_i - R_{i-1})\theta_R(Fo - Fo_i) + p_i\theta_p(Fo - Fo_i)]$$
$$m = 2, 3, \ldots, n \quad \text{and} \quad t_m < t < t_{m+1} \quad (4)$$

where $R_0 = Fo_1 = V_0 = 0$, and where $\theta_{ss}(Fo - Fo_i)_{\epsilon_i = Fo_i/Fm + \epsilon_1}$ represents the value of θ_{ss} which is obtained by the following substitutions of Fo and ϵ in Eq. 2.2.

$$Fo = Fo - Fo_i \text{ and } \epsilon = Fo_i/Fm + \epsilon_1$$

$\theta_{st}(Fo - Fo_i)_{\epsilon_i = Fo_i/Fm + \epsilon_1}$ represents the value of θ_{st} which is obtained from Eq. 2.3 through similar substitutions.

Determination of apparent thermophysical properties of warehouse stack

In order to predict the temperatures of a warehouse stack by use of the analytical solutions derived, the values of the following thermophysical parameters of the stack are required: Thermal diffusivity and Biot number. A computerized procedure was devised to estimate these values. Since it is rather difficult to estimate the values from the physical properties of component material, they are determined directly from an experimental temperature history curve at an internal location in the stack. Even though a heat transfer experiment in a warehouse is required to determine the parametric values, these values may be used for estimating the temperature response of warehouse stacks of various geometrical configurations at any internal location when they are subjected to various seasonal and/or daily variations in ambient air temperatures. The computerized method devised in the present investigation is described below.

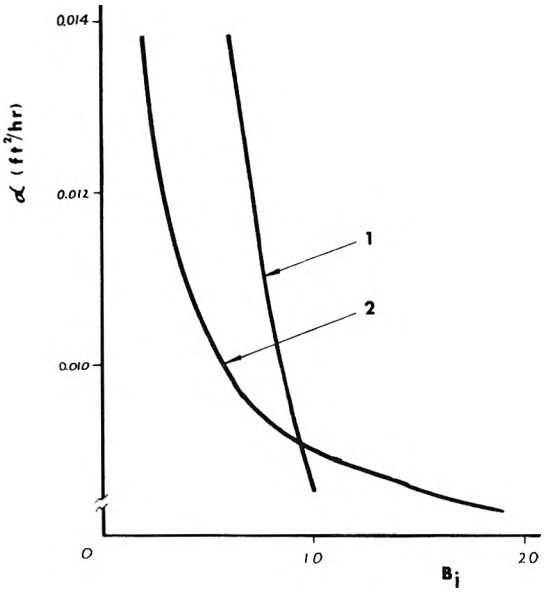


Fig. 4—Two curves used to estimate apparent Biot number and thermal diffusivity of a warehouse stack of canned tomato juice.

Temperature history curves of product at one internal location in a warehouse stack are estimated by using Eq. 4 and by using experimentally observed surrounding air temperatures in order to compare them with an experimental temperature history curve applicable to the same internal location. For this estimation, various Biot numbers and thermal diffusivity values in the following ranges are assumed: $0.1 \leqslant Bi \leqslant 100$ and $0.001 \leqslant \alpha \leqslant 0.1$ (ft^2/hr). From each temperature history curve estimated, one sum of square of differences between theoretically computed and experimentally observed temperatures is calculated. A thermal diffusivity value, which produces the minimum sum of square of differences, is then determined for each Biot number assumed, since a curved surface, which shows a relationship among Biot number, thermal diffusivity, and the sum of squares of differences, does not have a unique minimum point but has an infinite number of minimum points along a bottom line of a valley-like configuration. The simultaneous use of temperature history curves at more than one internal location does not give unique thermophysical property values since all computed curves of the sums of squares of differences are almost parallel to each other. Therefore, an additional criterion is used to determine these values. Surface conductance for naturally convective heating or cooling of the building wall is approximately equal to 2 Btu/(hr ft^2 F°) (Anonymous, 1972). Using the same conductance, the Biot number applicable to warehouse stack is:

Table 1–Constants used for estimating assumed air temperatures around warehouse stack of canned tomato juice (low level)

i	t_i(hr)	V_i(F°)	R_i(F°/hr)	P_i
1	0			−37.5
		2.90	−0.01825	
2	137			4.2
		3.00	−0.1370	
3	210			3.6
		3.00	−0.06444	
4	300			0.5
		2.25	0.05000	
5	330			0
		2.30	0.0	
6	400			

$$Bi = 2\ell/k = 2\ell/(\alpha cd)$$

or
$$\alpha = 2\ell/(Bi\ cd) \qquad (5)$$

When Eq. 5 is used together with the curve, which shows the relationships between thermal diffusivity values and the minimum sums of squares of differences in temperatures, we determine the two physical parameters of a warehouse stack. For a stack of canned tomato juice, we have: ℓ = 2 ft, c = 0.96 Btu/(lbF°), d = 48.67 lb/ft^3. The value of specific heat, c, is obtained from Dickerson (1968) by assuming that the specific heats and densities of air and paper-board are negligibly small when they are compared with those of tomato juice. The density, d, is estimated as:

$$d = (\text{ratio of void space}) \times (\text{density of tomato juice})$$
$$= 0.785 \times 62 = 48.67\ \text{lb/ft}^3.$$

For this estimation, we assume that the density of tomato juice is approximately equal to the density of water since the densities of all components except tomato juice have a negligible contribution to the estimation of the stack density. The ratio of void space is estimated through the geometrical analysis of a carton filled with the product.

Curve 1 in Figure 4 is calculated from Eq. 5. Curve 2 is obtained by applying the criterion on the minimum sum of squares of temperature differences. To estimate the sums of squares, we use temperature data, which are applicable up to 600 hr of storage. From the intersection of curves 1 and 2, we have:

$$Bi = 9.469 \quad \text{and} \quad \alpha = 0.009042\ \text{ft}^2/\text{hr}.$$

$$Bi = 2.725 \quad \text{and} \quad \alpha = 0.03928\ \text{ft}^2/\text{hr}.$$

The above values are obtained through the use of experimentally determined density since the shape of a glass jar is not geometrically simple.

The following thermophysical property values of a stack of jarred relish are also estimated through similar calculations.

Estimation of stack temperature

Temperatures at selected locations in warehouse stacks of sample products are estimated by using Eq. 4. A set of Fortran IV computer programs are prepared for this estimation because of lengthy calculations required. This set consists of programs for computing θ_{ss}, θ_{st}, θ_R, θ_p, and β_j as well as one for plotting computed temperatures in a chart-form by a Calcomp digital plotter.

Figure 5 shows typical results of a stack of canned tomato juice. To compute stack temperatures, assumed air temperatures are used. These assumed temperatures are obtained through mathematical analysis of experimentally measured air temperatures. Constants used for calculating the assumed air temperatures by Eq. 3.1 and 3.2 are given in Table 1. There is reasonable agreement between predicted and experimentally determined temperatures. It is observed that there are less than 10F° difference between the predicted and measured temperatures at locations examined during the initial period of storage. Predicted temperatures at the central location in the midheight level of the stack differ only slightly from measured temperatures, since the latter ones are used for estimating the thermophysical parameters of the stack. At 300 or later hours of storage, there are greater than 10F° difference between predicted and measured temperatures at a location midway from a

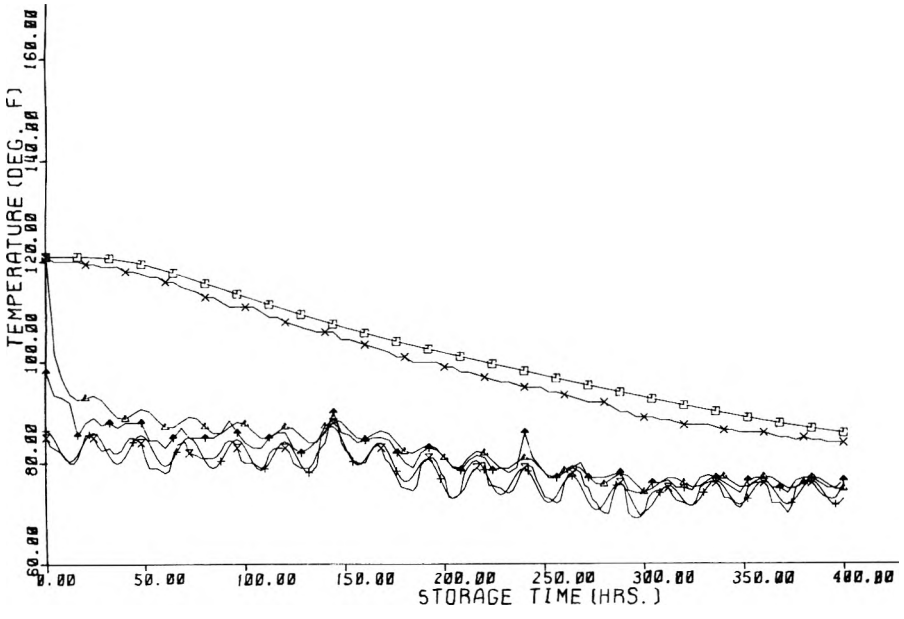


Fig. 5–Theoretically predicted and experimentally measured temperatures in the low level of a warehouse stack of canned tomato juice.

center to surface in a midheight level. There is better agreement between the two temperatures at all other locations tested.

Typical results for stack No. 2 of jarred relish are shown in Figure 6. Since there are no experimental temperatures collected after 48 hr and before 168 hr of storage, data points at these two storage times are connected with straight lines in these figures. As stated previously, the central temperatures of stack No. 1 collected up to 48 hr of storage are used for estimating thermophysical parameters. These parameters are used for calculating stack temperatures shown in the figure. There are 4F° difference between predicted and observed temperatures during an initial phase of storage and the difference increases to less than 8F° during the later period of storage.

DISCUSSION

IN THE PRESENT investigation, the theoretical formula, which is based on simple heat conduction in an infinite slab, is used for predicting transient temperatures in the sample warehouse stacks. There is reasonable agreement between predicted and measured temperatures as stated previously especially during an initial storage period.

The formula may be utilized for examining the temperature response of a warehouse stack, which is exposed to various climatic conditions provided Biot number and thermal diffusivity of the stack are known. Sample calculations are made for estimating transient state temperatures in the warehouse stack of canned tomato juice, which is thermophysically similar to that used in the present investigation. The initial temperature of canned tomato juice is assumed to be 100°F. Assumed ambient temperatures around the stack are represented by the following equations.

Hot temperature:	$T_{am} = 90 + 5 \sin (\pi/12)t$
Medium temperature:	$T_{am} = 65 + 5 \sin (\pi/12)t$
Cool temperature:	$T_{am} = 40 + 5 \sin (\pi/12)t$

Because of space limitations, temperature response charts obtained are not given in the present paper. At 100 hr of storage, the central temperature of the stack exposed to the hot air temperatures is lowered only by about 2F° and becomes approximately equal to 98°F. The central temperatures of stacks exposed to medium and cool ambient temperatures fall to about 90°F at the same storage time. The central temperatures of the stacks exposed to hot, medium and cool temperatures are down to 92, 70 and 50°F, respectively, at 400 hr of storage. The surface temperatures approach air temperatures fairly quickly although the larger the initial temperature difference between the surrounding air and product, the longer it takes the surface temperature to reach the air temperature.

When heat-processed food is stored in a severely cold climate, air in the warehouse is usually heated to prevent product damage due to freezing. Another computational test is made to examine the influence of failure in a heating system on product temperature. It is assumed that product and surrounding air are initially held at 40°F and that the center of sinusoidal changes in air temperature falls at a rate of 1F°/hr for the first 24 hr and maintains at 16°F after this time of storage.

Surface temperature falls to the freezing temperatures of product, 30 ~ 26°F, in about 3 hr after the surrounding air temperature reaches the upper limit of these temperatures. The air temperature reaches this limit at about 12 hr after a failure in the heating system. Therefore, food placed close to the surface of the stack likely initiates freezing unless the heating system is repaired within 15 hr in this assumed situation.

The thermal diffusivity value of a stack of jarred relish, which is determined in the present investigation, is considerably greater than that of canned tomato juice. The geometrical shape of the containers is likely the cause of this large difference. Since the relish jar used for our test has a barrel shape, there is likely less restricted convective air movement around the product during storage. Therefore, the apparent thermal conductivity of a stack of jarred relish is greater than that of a stack of canned tomato juice. Since a thermal diffusivity value is proportional to a conductivity value, the diffusivity of the former stack is greater than that of the latter stack. Biot number is inversely proportional to a thermal conductivity value according to its definition. Therefore, Biot number applicable to the stack of canned tomato juice is greater than that of jarred relish.

The equations obtained for predicting stack temperatures are fairly complex, although they are obtained by using a greatly simplified model for heat transfer; therefore, the equations are not suitable for manual computations. The computerized procedure, which is developed through the use of the

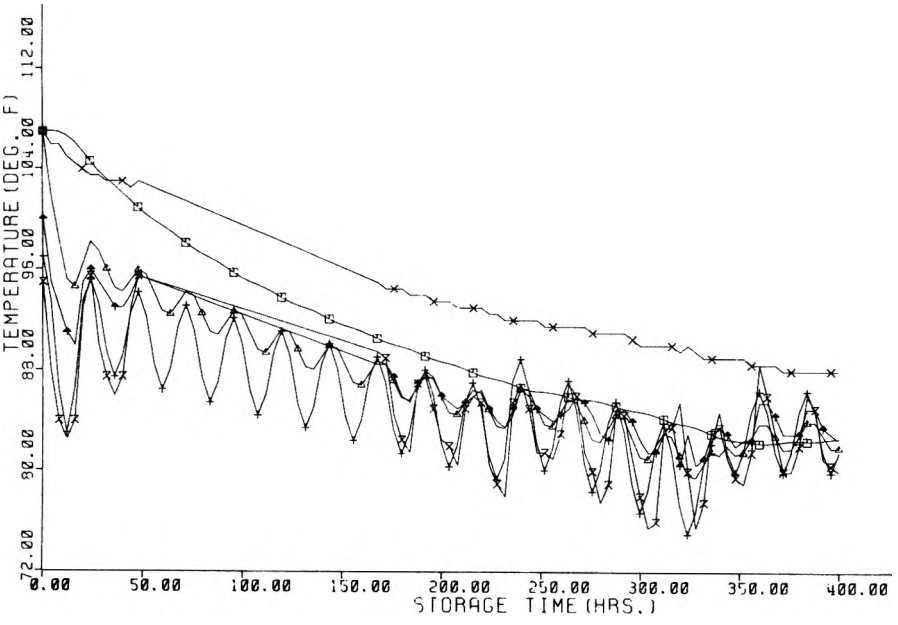
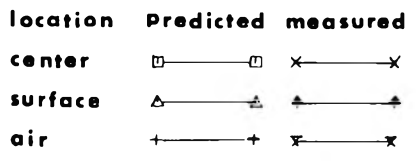


Fig. 6—Theoretically predicted and experimentally measured temperatures in the midheight level of a warehouse stack (No. 2) of jarred relish.



equations, requires less than 3 sec of computer time to estimate one temperature response curve when an IBM 360/Model 76 computer is used. This procedure will provide a valuable means for optimizing procedures for warehouse storage of heat-processed products.

CONCLUSION

A COMPUTERIZED procedure is developed for predicting the temperature response of a stack of heat-processed food, which is placed in a commercial warehouse shortly after production. This development is based on the careful examination of experimental data on the transient state temperatures of food in a warehouse stack and based on the application of a formula for heat conduction in an infinite slab. To estimate product temperatures by the computerized procedure, Biot number and thermal diffusivity of a stack are required. A method for determining these two physical constants is also developed. There is reasonable agreement between product temperatures determined experimentally and computed theoretically.

NOMENCLATURE

Bi = $h\ell/k$. Biot number (dimensionless).
c = Specific heat of warehouse stack [Btu/(lb F°)].
d = Density of warehouse stack (lb/ft^3).
Fo = $\alpha t/\ell^2$. Fourier number (dimensionless).
Fm = $\alpha/(\ell^2\omega)$. Modified Fourier number (dimensionless).
H = h/k (1/ft)
h = Coefficient of surface heat transfer [Btu/(hr ft^2 F°)].
k = Thermal conductivity of warehouse stack [Btu/(hr ft F°)].
ℓ = Half thickness of infinite slab or of warehouse stack (ft).
m = Dummy index integer
p = Magnitude of sudden increase or decrease in center of sinusoidal change in ambient air temperature. When there is a sudden increase in it, p is positive and when there is a sudden decrease, it is negative (F°).
R = Rate of linear changes in center of sinusoidal changes in ambient air temperatures (F°/hr).
T = Dimensional temperature (°F).
T_{am} = Dimensional ambient air temperature (°F).
t = Storage time variable (hr).
v = Amplitude of sinusoidal changes in air temperature (F°).
x = Distance measured from central vertical plane of warehouse stack or distance measured from central plane of infinite slab (ft).
α = Thermal diffusivity of warehouse stack (ft^2/hr).
ϵ = Initial angle of sinusoidal changes in ambient air temperature (Radian).
η = Expression defined by Eq. 2.14.
θ = Difference between temperature at any location in stack at any time of storage and uniform temperature of stack at zero time of storage (F°).
β_j = Constant obtained by solving the equation: $\beta \tan \beta = B_i$. The symbol represents the jth positive root of the equation.
θ_{am} = Difference between ambient air temperature at any time of storage and uniform temperature of stack at zero time of storage (F°).
θ_p = Expression defined by Eq. 2.5.
θ_R = Expression defined by Eq. 2.4.
θ_{ss} = Expression defined by Eq. 2.2.
θ_{st} = Expression defined by Eq. 2.3.
ξ = $\sqrt{1/2(Fm)}$ (dimensionless).
ρ = x/ℓ (dimensionless).
ϕ = Expression defined by Eq. 2.13.
ω = Angular velocity for sinusoidal change in ambient air temperature (Radian/hr). It is assumed that this velocity does not change during storage.

Subscript

i–ith value of quantity represented by symbol to which this subscript is applied. When it is used with p, it represents a value of p, which is applicable when $t = t_i$ or $Fo = Fo_i$. When it is used with either R, V, or ϵ, it represents a value of R, V, or ϵ, which is applicable to any t or Fo in this range: $t_i < t \leq t_{i+1}$ or $Fo_i < Fo \leq Fo_{i+1}$. Symbols t_i, T_{i+1}, Fo_i, and Fo_{i+1} are a specific dimensional time value, t, or dimensionless time value, Fo, at which there are changes in p, R, V, and/or ϵ. It is assumed that R, V, and/or ϵ do not change within these time limits: $t_i < t \leq t_{i+1}$ or $Fo_i < Fo \leq Fo_{i+1}$.

i+1, i–1–Similar to i.
m–Dummy subscript
n, n + 1–Similar to i. Symbol, n represents also the number of sets of equations which are required to simulate one ambient air temperature history curve. Symbol n + 1 is used as t_{n+1}, which represents the maximum time value up to which stack temperatures are to be computed.
o–used as R_o and V_o, which are zero quantities.
1, 2–Similar to i. t_1 represents zero time of storage.
ϵ = o–Used as $\theta_{st, \epsilon = o}$, which represents the value of θ_{st} obtained by inserting ϵ = o into Eq. 2.3.
$\epsilon = \pi/2$–Used as $\theta_{st\epsilon = \pi/2}$, which represent the value obtained by substituting $\epsilon = \pi/2$ into Eq. 2.3.
ϵ_i = Fo/Fm + ϵ_1–Values of θ_{ss} and θ_{st}, which is obtained by substituting $\epsilon = Fo_i/Fm + \epsilon_i$ into Eq. 2.2 and 2.3, respectively. ϵ_1 is the initial angle at $Fo = Fo_1 = 0$.

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Ms received 6/19/75; revised 11/1/75; accepted 11/5/75.

Presented at the 35th Annual Meeting of the Institute of Food Technologists, Chicago, Ill., June 8–12, 1975.

A paper of the Journal Series, New Jersey Agricultural Experiment Station, Cook College, Rutgers University, The State University of New Jersey, P.O. Box 231, New Brunswick, NJ 08903.

Contribution No. 447 from Engineering Research Service, Agriculture Canada, Ottawa, K1A 0C6, Canada.

This work was supported in part by U.S. Public Health Service Grant No. FD 00119 from the Food and Drug Administration.

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INFLUENCE OF PROCESSING VARIABLES ON FLAVOR STABILITY OF PEAR PURÉE

ABSTRACT

Whole Bartlett pears at the optimum stage of ripeness were processed into purée using an enclosed system which facilitated the inactivation of enzymes, the separation of skins, seeds and fibers, and the capturing of pear volatiles which would be lost in conventional open systems. When added back, the captured essence significantly enhanced the flavor of the purée. Purées containing 50% inherent essence add back were more acceptable than purées which contained 0 or 100%. Using whole pears in a bulk system yielded 77% more pear purée than the system in which pears were peeled and cored before processing. Flavor differences between canned purées prepared in open and closed systems were readily detectable. More flavor and better color were observed in purées canned without than with sucrose. Little flavor difference was observed between purées processed by aseptic and hot-filling methods. No significant flavor losses occurred in the canned purées during the first year of storage.

INTRODUCTION

LARGE QUANTITIES of sound pears with desirable flavor do not meet requirements for canned pear halves because of irregular size, shape or surface blemishes. These fruits are utilized in products such as pear nectar, purée or concentrate. In the commercial preparation of these pear products, little effort is directed toward retaining the volatile components of the fruit. Generally, processing is accomplished in open systems in which the necessary heating drives the pear volatiles off into the atmosphere. The resulting product has such low flavor intensity that it is sometimes mistaken for applesauce.

The late Carl Rietz developed the Thermopulper (Rietz Mfg. Co., Santa Rosa, Calif.) which was designed to inactivate enzymes and to pulp whole fruits in a completely closed system. The enzymes are inactivated with heat at a slightly positive pressure. By modifying the Thermopulper, separation and recovery of the pear volatiles became feasible. The system which facilitated both essence separation and the removal of peels and cores of whole pears was described by Jennings et al. (1960).

The present investigation was undertaken to determine the influence of closed and open processing systems, the effect of bulk handling of whole pears, the effect of essence addback, and other processing variables on purée flavor, acceptability, and yield. Six processing variations are discussed.

METHODS & MATERIALS

Raw material

Bartlett pears at approximately 17 lb pressure were harvested and stored at 32°F (0°C) for 3 wk to assure even ripening. The pears were ripened at 68°F (20°C) and 85% RH to an average pressure test of 2 lb, measured with a Magnus-Taylor pressure tester equipped with a 5/16-in. plunger.

Processing

The fruit was spray-washed and culls were discarded. When needed, a Model 6-L Pear Preparation Machine (FMC, San Jose, Calif.) was used for peeling and coring pears. The system used to crush, heat and pulp the fruit consisted of a Thermopulper, a holding section, a Langsenkamp EZ Adjust Micro Vac Pulper equipped with an 0.060 screen and a Langsenkamp EZ Adjust Micro Finisher equipped with an 0.027 screen. The Thermopulper, essentially an inclined steam screw, was operated at 215°F (101.7°C). The product could be heated by both direct steam injection and conduction. The heated macerated product was passed into a horizontal holding section where the temperature was maintained at 212°F (100°C). The feed rate was 22 lb pears per min and the approximate residence times in the Thermopulper and holding sections were 10 and 7.5 min, respectively. From the holding section, the product was pumped to the pulper and finisher where skins, seeds and core material were removed. The purée was cooled to room temperature in a Creamery Package Heat Exchanger.

Heinz et al. (1964) established that pear essences derived by flash (deaerator) techniques possess more desirable aroma than by reflux methods. However, at the time of the experiment, the equipment was not available and the reflux (Oldershaw) technique was used to capture the pear volatiles during processing. This means that the vapors from the inclined steam screw and holding section were recovered through three condensers attached in series. The condensates were collected and weighed. The first condenser was cooled with tap water, 76–68°F (24.4–25.6°C), the other two were cooled with iced water, approximately 40°F (4.4°C).

Except in Study 3, essence from the second and third condensers was restored to half of the purée in direct proportion to the amount of purée from which it was collected. This purée represented the product which would result from pears processed in a closed system. Condensate from the first condenser was added to adjust the solids content of the purée to that of the fresh pears. To the other half of the purée, water was added to obtain equivalent soluble solids concentration. The resulting purée was representative of product prepared in an open system. Except for the listed variations, the purées were prepared with 5% sucrose by weight and aseptically canned.

For canning, the purée was heated to 230°F (110°C), held for 10 sec, cooled to approximately 100°F (37.8°C), then aseptically filled into presterilized 202 × 214 plain tin cans and steam-flow closed in a Dole Aseptic System.

Studies 1–6

1. Open vs closed systems of processing. It was assumed that restoration of no essence to the purée would represent an open system, whereas returning 100% of the captured essence represented a closed system.

2. Whole vs peeled pears. Purées from peeled and whole fruits were compared to determine whether the peel and core materials retained during heating contributed to pear flavor, product quality and yield of purée. One lot of pears was mechanically peeled and cored before processing and the other lot was processed whole.

3. Restoration of pear essence. Purées containing 0, 25, 50 and 100% of the inherent essence were compared to determine the influence of essence restoration on flavor intensity and acceptability. The pear purée was completely stripped by heating to 230°F (110°C) in a Creamery Package Heat Exchanger and concentrating in a 3-stage flash evaporator. The product was recycled through the heat exchanger and evaporator until a concentration of approximately 24° Brix was obtained. The concentrate was divided into four parts. The captured essence was restored to the concentrate and water was added to the 0% (control), 25% and 50% essence samples to equalize the solids levels.

4. Influence of sucrose. To determine whether sucrose affected flavor retention during canning and storage, samples canned with 5% sucrose were compared with those that were canned without sucrose.

[1] Deceased

5. Aseptic processing vs hot filling. One part of the purée was aseptically canned as described. The other part was heated to 196°F (91.1°C) and hot filled into unsterilized cans. The closed cans were inverted, held for 3 min to sterilize the can tops and cooled in chlorinated water.

6. Storage time and temperature. Samples were evaluated after 1, 2, 4 and 12 months of storage at 32° and 68°F (0° and 20°C).

Sensory evaluation

Ten judges were selected based on their previous training and experience on pear aroma panels. Prior to each evaluation period, two orientation sessions were spent evaluating commercial samples of canned pear purée. Judging was conducted daily between 9:30 and 10:30 a.m. in individual, partitioned booths, maintained at 70° ± 1°F (21° ± 1°C). Red illumination of low intensity masked visual differences. Samples were presented at room temperature in coded, 50-ml beakers. Distilled water was provided for oral rinsing, and the judges were instructed to swallow only a small portion of each sample. Two replications per judge were obtained.

At "O-time" (1 wk after processing), sets of four samples representing the variables for the raw material processed in both open and closed systems were evaluated by multiple comparison. Judges scored flavor intensity (based on a 9-point scale where 8 = high flavor and 0 = no flavor) and ranked acceptability (1 = most desirable and 4 = least desirable). Qualitative flavor descriptions were also recorded.

After each storage period, the samples were evaluated by scoring flavor intensities using a streamlined, 7-point scale where 6 = high flavor, and 0 = no flavor. One set of samples was stored at room temperature, 68°F (20°C) and the other at 32°F (0°C).

In addition, at "O-time," triangle tests were made to determine whether the panel could differentiate between purées made from whole and peeled fruits.

Chemical and physical analysis

The data are the averages of determinations on six randomly selected cans. Soluble solids, pH, and total acidity (percent by weight anhydrous citric acid) were determined using the methods of AOAC (1970). The color of the purées was measured with a Gardner Color Difference Meter using the LY_2 reference color plate (Rd = 61.3, a = −1.9, b = +22.8).

RESULTS & DISCUSSION

Open and closed systems of processing

The results of multiple comparison sensory evaluation indicated significantly higher flavor intensity in purées processed in the closed system made from either peeled or whole pears (Table 1). Although flavor intensity was scored consistently higher, no significant preference (lower avg rank) was indicated for purées processed in a closed system, containing 100% of the inherent pear volatiles.

The pH and total acidity values (Table 4) were also similar. The difference (0.9°B) in soluble solids value of purées from whole and peeled pears is primarily the result of handling the peeled and cored pears in water from peeling to processing. The procedure prevented the browning of peeled and cut surfaces but caused some loss in soluble components. Variations in the raw material account for most of the differences in the analytical data.

Purées from peeled and whole fruit

Although multiple comparison analyses showed no flavor differences between purées from whole or peeled pears, by triangle testing, the judges correctly distinguished between whole and peeled samples from the open and closed systems in 14 and 16 out of 20 evaluations, respectively. These results are significant ($p < 0.01$). The flavor of purées made from peeled and cored pears was considered more intense than that from whole fruit yet the preference for the purée was not significant. Changes in flavor intensity through storage are shown in Figure 1.

Although flavor acceptability was not better, there is a definite economical advantage to processing whole rather than peeled pears. The machine used in the experiment cut away the peel, halved the fruit and spooned out the core material,

Table 1—Influence of processing methods on flavor intensity and acceptability of pear purée using multiple comparison sensory evaluation (0-time)

	Closed system		Open system	
	Peeled pears	Whole pears	Peeled pears	Whole pears
Avg flavor intensity[a]	5.6a	5.4a	4.7b	4.2b
Avg rank (acceptability)[a]	2.3a	2.6a	2.4a	2.7a

[a] Within each row, values having a letter in common do not differ at $p < 0.05$. ($n = 20$).

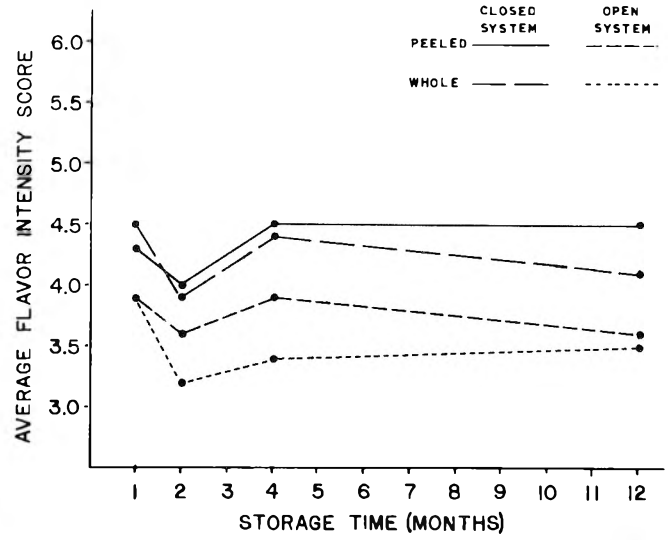

Fig. 1—Influence of storage time on flavor intensity of pear purées processed in open and closed systems from whole and peeled/cored pears.

causing losses totaling 48% by weight of the raw pears. Using better procedures would reduce peeling and coring losses but not so to the level which is obtained by processing the whole fruit. Hart et al. (1970) used a 20-sec lye dip and soft rubber discs to remove the peel, reporting peeling losses as low as 14–20% (coring losses not included) depending on pear maturity. In manufacturing pear nectar, 30–35% peeling and coring losses were reported for Bon Chretion pears in South Africa (Anon., 1973). These investigators discussed the need of recovering and using the pear juice from the peel and core material.

Producing purée by the process described in this work, peeling and coring wastes were minimized by processing the whole pears. Although pulping and finishing losses were 10% by weight for whole pears as compared to 1% for peeled and cored pears, 77% more purée was produced when the pears were cooked and pulped whole (Table 2).

The flavor quality of the purée made from whole pears was comparable to that produced from peeled and cored fruits and had brighter color, as measured by the Gardner Color Difference meter (Table 5).

Influence of essence concentration on flavor intensity and acceptability of pear purée

In multiple comparison flavor evaluation of the purées with 0, 25, 50 and 100% of the inherent essence restored, intensity

Table 2—Processing data for purées from whole and peeled pears

	Whole pears (lb)		Peeled pears (lb)	
Whole pears (Raw material)	1854		1840	
	Yield	Losses	Yield	Losses
Peels and cores		–		885
Pear purée	1416		879	
Condensates first	186		37	
second	69.5		20	
Pulper discharge		50.5		1
Finish discharge		132		18
Total yield	1671.5		936	
Total loss		182.5		904

Table 3—The influence of essence addback on flavor intensity and acceptability of canned pear purée

	% of Inherent essence restored			
Treatment	0	25%	50%	100%
Avg flavor intensity[a]	3.5a	4.5b	5.2b	6.1c
Avg rank (acceptability)[a]	2.9b	2.4ab	2.0a	2.8b

[a] Within a row, values having a common letter do not differ at $p < 0.05$. ($n = 20$).

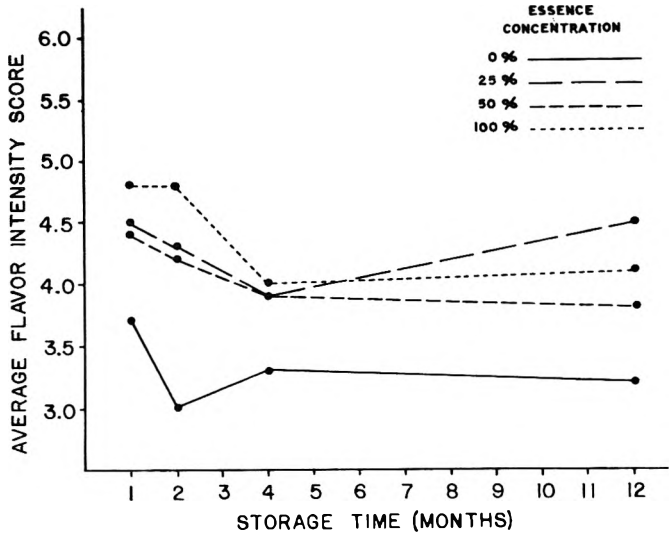

Fig. 2—Influence of storage time and essence restoration on flavor intensity of pear purée.

scores increased with increasing essence concentration (Table 3). The influence of storage on flavor retention is shown in Fig. 2.

Acceptability did not correspond to increasing flavor intensity. The purée with 100% restored essence was judged too intense. The purée with 50% essence was significantly more acceptable than the control (0%) and the fully restored (100%) purée, both of which received similar acceptance. This might explain why the panel did not prefer either purée processed in a closed or open system; the flavor was either too intense or too weak.

Purées canned with and without sucrose

Initially, flavor differences were readily apparent between purées from open and closed systems of processing. Average flavor scores were 1.1–1.3 points higher for purées produced in the closed system. Lower flavor intensity was ascribed to samples which were canned with sucrose as compared to samples to which sucrose was added after processing (Fig. 3). Samples with greater flavor were judged to be more acceptable. Purée canned without sugar had significantly brighter color which the samples maintained during one year of storage (Table 5). The addition of sucrose at canning seemed to reduce both flavor and color qualities.

Aseptic and hot-fill canned purées

Insignificant differences in flavor were detected between the purées canned by the two procedures. Except for the hot filled purée (closed system) stored at room temperature, neither the canning procedure nor the temperature of storage showed significant influence on flavor.

Color differences measured by Gardner Color Difference Meter showed significantly greater brightness in the aseptically-canned purées (Table 5). As in other tests, there were distinct flavor differences between the open and closed systems of processing. The purée produced in the open system averaged 1.7 flavor points lower than the purée from the closed system (Fig. 4).

Table 4—Chemical analyses of pears and canned purée

Samples	Pressure test (lb)	0 Time pH	0 Time Total acidity % anhyd. citric acid	0 Time Soluble solids °Brix	1-yr storage (20° C) pH	1-yr storage (20° C) Total acidity % anhyd. citric acid	1-yr storage (20° C) Soluble solids °Brix
Raw material	1.9	4.01	0.287	14.0	–	–	–
Purée from peeled pears		4.00	0.231	13.4	3.98	0.227	13.4
Purée from whole pears		4.05	0.243	14.3	3.93	0.239	14.2
Raw material	2.0	4.14	0.319	14.4	–	–	–
Purée canned with sucrose		4.15	0.213	18.2	4.08	0.210	18.3
Purée canned without sucrose		4.12	0.242	13.5	3.95	0.239	13.8
Raw material	2.1	3.87	0.298	14.4	–	–	–
Aseptically canned purée		3.90	0.266	13.9	3.83	0.248	13.9
Hot-fill canned purée		3.91	0.265	14.0	3.90	0.246	13.9

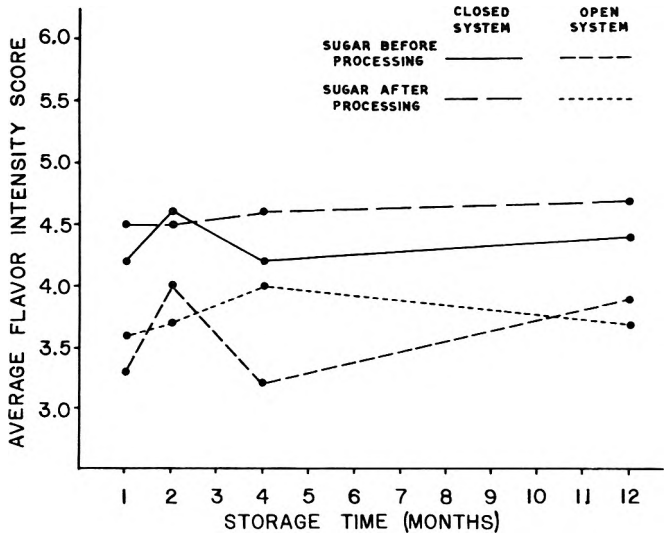

Fig. 3—Influence of storage time on the flavor intensity of pear purée processed in open and closed systems and canned with and without 5% sucrose.

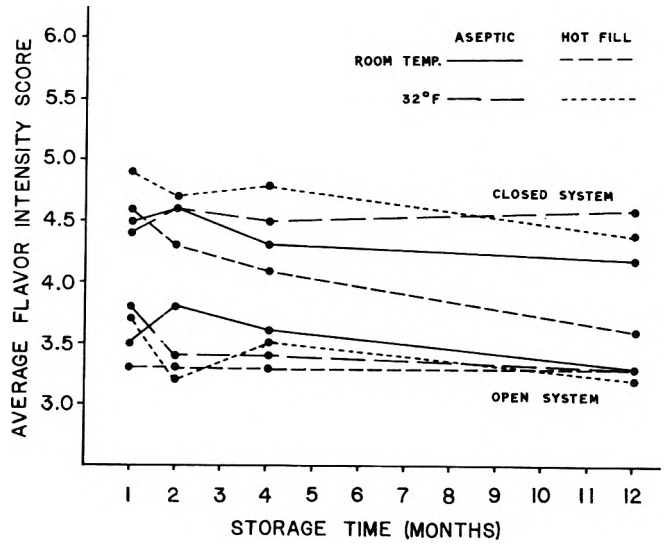

Fig. 4—Influence of storage time and temperature on flavor intensity of pear purée processed in open and closed systems and canned by aseptic and hot-fill methods.

Influence of storage time and temperature on flavor retention

In general, temperature and time of storage up to 1 yr had no significant influence on flavor intensity and acceptability (Fig. 1, 2, 3 and 4). Only the hot filled purée processed in the closed system showed a progressive flavor loss (one flavor point) during storage at room temperature (Fig. 4).

Insignificant chemical changes occurred after 1 yr storage (Table 4). Luh and Sioud (1966) showed decrease in pH and increase in titratable acidity as a result of storage. Luh and Sioud (1966) also related storage time and hydroxymethyl furfural formation to the browning of pear purée. Browning, as measured by the Gardner Color Difference Meter, primarily influenced the "Rd" and "a" values of the purée color, causing decrease in "Rd," increase in the "a" values. In this experiment, little change in "Rd" values occurred, and "a" values were observed to decrease with storage (Table 5.). These changes made the samples with lower "a" and "b" values, the purée prepared from peeled and cored pears and the purée which was canned with sugar, appear green at "O-time" and greener after a year of storage. For the other samples, storage seemed to have improved color in relationship to the reference color plate.

CONCLUSION

THE DATA INDICATE that processing pears in a closed system significantly increased flavor intensity which persisted through 1 yr of storage. Purées with 50% essence addback were more acceptable than the purées to which the essences were fully restored.

The results of this research also indicate the feasibility of using whole pears in the production of pear purée and similar pear products. By using whole pears, peeling and coring losses

Table 5—Influence of storage time on the color of pear purée, as measured by the Gardner Color Difference Meter

	0 Time			1-yr storage (20°C)		
	Gardner values			Gardner values		
Purée Samples	Rd[a]	a[b]	b[c]	Rd[a]	a[b]	b[c]
From peeled pears	26.7	−2.0	14.7	26.6	−4.2	15.8
	***	***	***	***	***	***
From whole pears	29.6	−0.5	20.5	29.8	−1.1	21.8
Canned with sucrose	28.1	−1.5	20.3	27.8	−2.3	20.7
	***			***	***	***
Canned without sucrose	30.3	−1.3	20.4	30.0	−1.7	21.6
Aseptically canned	28.0	−0.02	19.6	28.9	−0.4	21.9
	***			***	*	
Hot-fill canned	27.3	0.03	19.7	27.5	−0.3	21.3

[a] Brightness, higher value = brighter.
[b] Green-red value, lower value = greener.
[c] Yellow-blue value, higher value = yellower.
* Significant at $p < 0.05$
*** Significant at $p < 0.001$

were significantly reduced which resulted in a 77% higher yield and minimum waste. The purée from whole pears was as acceptable as purée from peeled and cored pears.

The flavor of the pear purée was primarily influenced by the addback of essence. Canning the purées with sugar appeared to be detrimental to both color and flavor. The aseptic method of canning significantly influenced the color but not the flavor. At both 68°F (room temp) and 32°F, the color of the purées became brighter with storage. The flavor of the purées did not change significantly in a year at either storage temperature.

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Ms received 9/30/75; revised 12/19/75; accepted 12/25/75.

The studies were supported, in part, by funds donated by Pear Zone No. 1, San Francisco and the Sugar Research Foundation, Inc., New York.

The assistance and encouragement of Dr. W.G. Jennings throughout these studies is greatfully appreciated. Appreciation is extended to Mr. D.N. Stewart, Mrs. Sandra Gee, Mrs. Teri Wolcott and J.R. Heil for technical assistance.

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METHODOLOGY FOR DIRECT CONTACT FREEZING OF VEGETABLES IN AQUEOUS FREEZING MEDIA

ABSTRACT

This work treats the freezing of foodstuffs in aqueous media with the intention of reducing the pickup of solute on the solid. Product frozen in 23% NaCl solution (AF 23-0) was centrifuged, washed, and/or blotted to remove surface adhering freezant. Freezing times of less than 1 min were obtained for peas, diced carrots, snow peas and cut green beans. This corresponded to an estimated surface heat transfer coefficient in the range 50–150 BTU/hr ft²°F. Salt residuals ranged from 0.48% (peas) to 2.2% (carrots) and compared favorably to the values reported for canned and remanufactured products. Preliminary cost analysis indicated competitiveness of the method to air-blast freezing methods.

INTRODUCTION

FREEZING METHODOLOGY using air, cryogenic gases, direct immersion cryogenic fluids and direct immersion aqueous fluids has been extensively studied (Tressler et al., 1968; Fennema, 1973). Freezing in each of these heat transfer media has recognized disadvantages. For instance, freezing in cold air (convective heat transfer) requires large-sized equipment because of the low heat-transfer rate. Relatively high heat-transfer rates can be achieved by rapid circulation of the air past the product, but this leads to excess energy consumption. Based on 1969 European costs, fan power consumption accounted for approximately 8% of the total cost of freezing and 25% of all energy costs (Romijin, 1969). Considering the rapidly rising cost of energy (Ludwig, 1975), increases in these percentages can be anticipated. Excess energy also must be provided in the form of additional refrigeration capacity to remove heat generated by the movement of air within the freezer chest. Finally, freezing in cold air often leads to product dehydration caused by the water vapor pressure differential between the cold air and warm food.

When the heat-transfer fluid is a liquid such as freezant 12 (boiling and convective heat transfer) high operation costs result due to losses of the heat-transfer medium. Losses of 1–2.0% are not uncommon (Lawler and Trauberman, 1969; Moser, 1969). Specialized and costly equipment is required for vapor recovery to minimize losses. Moreover, there is speculation that freezant 12 presents a health hazard due to its contribution to depletion of ozone in the stratosphere (Cicerone et al., 1974; Anon., 1974). Freezing in a liquid cryogen such as liquid N_2 also requires excess power for circulation of vapors since nearly half of the heat removed by this medium is removed by convective heat transfer from the cold vapor phase (Dinglinger, 1969).

When the heat-transfer fluid is an aqueous medium, such as a sodium chloride solution, the uptake of solute by the product and subsequent solute related flavor changes and the requirement for periodic replacement of the medium can be disadvantages. However, aqueous freezants (AF) have distinct advantages to be considered in spite of possible flavor drawbacks. For instance, AF feature high heat transfer rates (Mott, 1964) low cost, and potential safety. Moreover, AF inherently reduce product dehydration and coil frosting problems associated with air-blast freezing.

This report evaluates aqueous freezing as an alternative to current freezing methodology. The text illustrates the heat transfer characteristics of an experimental system, identifies solute uptake mechanisms, and prescribes and evaluates methodology for reducing solute uptake. The uptake of the aqueous freezant by the food material is the primary problem in the development of this system and will be considered first.

Solute uptake: Major mechanisms and prescribed methodology

Three major mechanisms contribute to solute uptake during a direct contact immersion freeze. These are bulk uptake or occlusion by absorption into partially dehydrated tissue, infusive uptake acting under the influence of a solute concentration driving force, and bulk uptake by adherence of the viscous transfer medium to the porous product surface. Indeed, the relative importance of each mechanism is a function of product composition, product shape, and product porosity. However, the extent of solute pick-up by each mechanism can be controlled by judicious selection of the processing conditions before and after the freeze immersion.

Absorption. Bulk uptake or occlusion by partly dehydrated tissue can be reduced by maintaining the product moisture balance during the pre-freeze blanching and cooling processes. The moisture balance would be favorably maintained during the blanch followed by water cooling, and little, if any, solute could be added by this mechanism during the freezing immersion. Conversely, water lost by dehydration during a post-blanch air-cooling period would be replaced, at least in part, by solute-bearing fluid.

Infusion. Uptake via infusion and surface adherence can be reduced by attention to the design and operation of the immersion freezer itself. Solute uptake occurs during the cooling and ice formation process by infusion or diffusion of the solute solids into the tissue of the product being frozen. Infusion occurs more slowly in solids than in liquids and can be reduced greatly by an ice barrier on the exposed product surfaces. Rapid ice barrier formation and minimum solute uptake by infusion can be obtained by maximizing the temperature differential and heat transfer coefficients between the solution and food pieces and minimizing the mass transfer coefficient. The temperature driving force is maximized by use of heat-transfer liquid at as low a temperature as possible. Heat transfer coefficients are maximized by the use of mechanical turbulence in the transfer liquid. However, since solution agitation increases the mass transfer coefficient, the relative changes in the mass and heat transfer must be determined experimentally.

Entrainment. Bulk solute uptake entrainment or adherence on the surface of the frozen product as it leaves the immersion bath is influenced by product surface roughness, continuity and wettability, and by solution viscosity. Solute uptake by entrainment can be minimized by a dilute freezant solution since low solute concentration heat-transfer liquids have lower

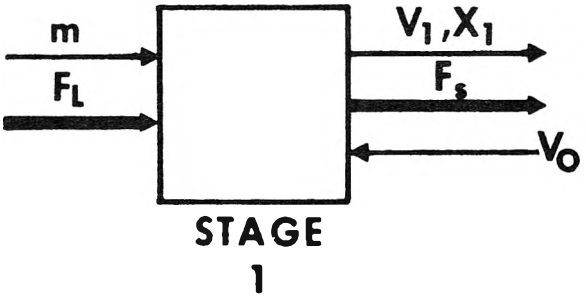

Fig. 1—Flow schematic for single-stage freezing.

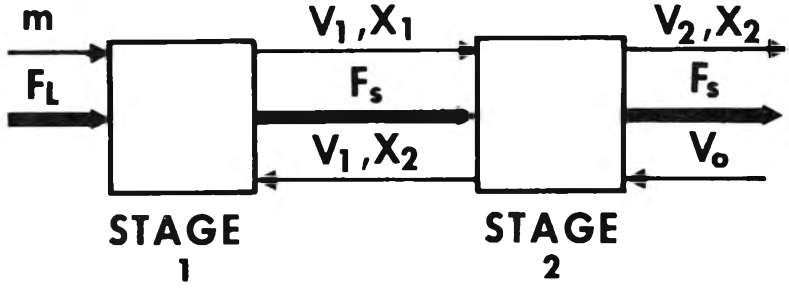

Fig. 2—Flow schematic for two-stage freezing.

viscosities as well as reduced solids content. These liquids have a higher freezing point and cannot provide as great a temperature differential as the more concentrated freezants.

Therefore, reduction of solute uptake to acceptable levels requires a compromise between the requirement of high solute concentration for low freezing temperature and rapid ice formation, and the requirement of low solute concentration for reduced solute entrainment. This compromise can be reached by freezing the food (fruit or vegetable) in a concentrated low temperature solution and then rapidly washing in one or more dilute solutions maintained at temperatures between the freezing point of the medium and the product. We have named this technique sequential countercurrent aqueous freezing (SCAF).

The anticipated concentration of entrained liquid on washed and on unwashed products can be determined from a mathematical model. Figure 1 illustrates schematically the material flow for a single-stage aqueous contact freezer with no washing. The mass flow of food through the freezer is invariant and is indicated by F_L for the unfrozen state and F_S for the frozen state. Solute addition is at mass rate m, solvent addition at volumetric rate V_O, and loss of solute by entrainment on the product is indicated at a volumetric rate v_1 and concentration x_1 (g solute/g entrained solution). Figure 2 illustrates a two-stage freezer with freezing (stage 1) and washing (stage 2). Material flows are arranged so that the entrainment volume v_2 leaving stage 1 at concentration x_1 is replaced by an equal volume of heat-transfer fluid from stage 2 at concentration x_2. Furthermore, the entrainment volume v_2 leaving stage 2 at concentration x_2 along with the final product is replaced by a volume V_O of solute-free solvent. Solid solute is added to stage 1.

Based on a solute material balance for a two-stage system, the solute concentration associated with product leaving the second stage is

$$x_2 = \frac{1/v_2}{1/v_1 + 1/v_2} \cdot x_1 \qquad (1)$$

The analysis can be extended to N-1 washing stages in a N-stage SCAF as illustrated in Figure 3. The concentration x_n of entrained solute leaving with the product from any stage n in the sequence is

$$x_n = \frac{\sum_{i=n}^{N} 1/v_i}{\sum_{i=1}^{N} 1/v_i} \cdot x_1 \qquad (2)$$

For the special case of constant overflow, i.e., $v_1 = v_2 \ldots v_n = v_N$,

$$x_n = \frac{N - n + 1}{N} \cdot x_1 \qquad (3)$$

and the concentration leaving the last stage is

$$x_N = \frac{x_1}{N} \qquad (4)$$

Consequently, the concentration of solution (x_N) entrained on the frozen product leaving the last stage of the system in a two-stage, three-stage or four-stage process is respectively 1/2, 1/3, or 1/4 of that in a one-stage process. Lower surface concentrations than predicted by Eq (4) could be achieved by washing directly in cold water, but this would create a solution disposal problem.

In summary, the SCAF process uses an initial low temperature freezing stage followed by countercurrent "washing" stages using dilute freezants at temperatures below the freezing point of the food. The SCAF achieves a theoretical reduction of solute uptake with no effluent. "Spent" freezant leaves the system with the product.

The mathematical analysis described above may be applied to specific solutes to establish operating conditions for the freezing step. Table 1 shows the predicted steady-state operating conditions for several aqueous freezing media. Stage 1, the freezing stage, is at the eutectic concentration in each example and provides the most rapid available freezing.

Bulk solute taken up by adherence to the product surface can be reduced by post-freeze or post-wash (when the SCAF is also utilized) mechanical treatments. Centrifuging of unrestrained liquid is one approach. Another method is sponging or blotting with an absorbent material.

MATERIALS & METHODS

SCAF was experimentally examined in a model system using aqueous solutions of sodium chloride. Sodium chloride was selected because of its historical place in food immersion freezing and because of its flavor, low cost, and ease of analysis. The solution used as the freezant was 23% NaCl (AF 23-0) and the solution used as washing liquor was 11.5% NaCl. These concentrations were selected to match the steady-state concentrations of the two-stage SCAF described earlier. No attempt was made to match overflow volumes from stage-to-stage since the amount of product frozen was small and the reservoir volumes were large. Blotting and centrifugal removal of solute were also tested.

Products chosen for freezing represented several vegetable tissue types and geometries. These were peas (uncut starchy seeds in spherical form), carrots (cut root tissue in cubical form), beans (cut and uncut pod and seed tissue in cylindrical form) and snow peas (uncut pod tissue in flat plates). Product preparation followed recommended industrial practice. The time between the wash and blanch was longer than usually used.

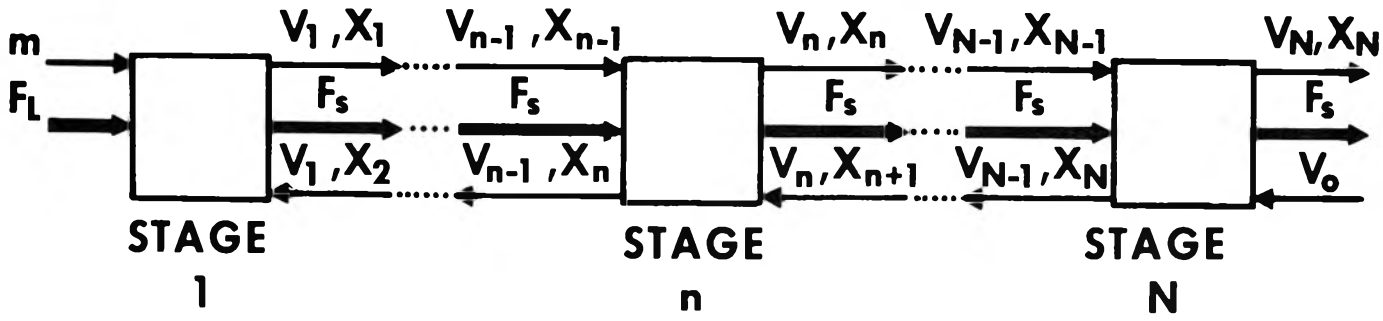

Fig. 3–Flow schematic for N-stage freezing.

Produce used in these experiments was obtained at a local fresh-produce market and was of Mexican or Californian origin. Carrots (unknown variety) were diced (nominal 3/8 in. cuts). Peas (Bamoa variety), snow peas, and snap beans were in field condition: i.e., untrimmed or unshelled.

The freezing unit consisted of the insulated freezing cabinet and the necessary refrigeration machinery, transfer pumps, and freezant storage reservoirs. The principal element of the freezer cabinet was a perforated drum (8.0 in. diam by 7.4 in. long). Perforations were 3/16 in. diam on 1/4 in. staggered centers. The drum surface presented about 51% open area. The drum could be rotated at speeds up to 200 rpm around the cylinder axis (see Fig. 4). One face of the drum was removable for product loading or unloading.

Heat-transfer fluid could be maintained to submerge the rotating drum to a maximum depth of 2.0 in. (measured from the cylinder inside surface to the liquid surface). The fluid level could be lowered in less than 5 sec to allow draining and centrifuging free of the liquid surface.

A 5/8 in. by 8.0 in. baffle was mounted on the inside surface of the drum to promote turbulence in the heat-transfer fluid and increased product submersion and product mixing.

Freezant fluids were pumped to the freezing cabinet and sprayed through a 1/4 in. pipe directly onto the revolving drum and through the perforations onto the floating product. Freezant fluid returned to the reservoir by gravity. Freezant was recirculated at 16.0 lb/min.

The freezant liquor (AF 23-0) was maintained at 0 ± 2°F and the wash liquor (11.5% NaCl) within 2°F of set point temperature. Reservoirs were polyethylene or stainless steel tanks, and heat-transfer surfaces were stainless steel (type 316).

Wash fluids were applied by full immersion as above but without spraying, or were poured over the revolving drum (50 rpm) in simulation of a cascade over the product. Cascade rate was 0.46 lb/sec for a 10 sec duration.

The product blotting apparatus (Fig. 5) consisted of a pair of 2.0 in. diam compression rollers and four 1/2 in. thick, 1.2 lb/ft^3, open-cell polyurethane blankets. Frozen product was distributed on the surface of one blanket and then passed between the compression rolls sandwiched with two blankets above and two blankets below. The blanket sandwich was compressed into a 3/4 in. thickness between the compression rolls.

Product was blanched in a stainless steel basket contained in a steam chest.

Procedures

Undiced carrots were diced the morning of use, graded, washed and stored in an insulated container at 60°F. Peas were shelled, washed, graded and stored in an iced container at 32°F. Snow peas were destemmed, washed, iced and stored. Beans were cut (1.0 in. nominal lengths), washed, iced and stored.

At the beginning of each run, the required amount of product (100g) was weighed, blanched, cooled and reweighed. Product was blanched in steam at 210°F for 3.0 min. Product was cooled in water at 57 ± 2°F for 1.0 min and drained for 0.75–1.0 min. Alternatively, product was cooled in air for 1.5 min.

During the blanching and cooling sequence, the freezant level in the freezer cabinet was established. The product was then placed in the drum, the transfer pump started, and the freezer drum rotated at 50 rpm. At the end of the freezing period, the freezant level was lowered, and drum rotation stopped to allow draining for 15 sec. The product was then centrifuged at 200 rpm (4.5 × G).

For "washing" experiments, the drum rotation was next adjusted to 50 rpm and 4.6 lb of washing liquor was poured over the drum at a rate of 0.46 lb/sec. The product was again drained (15 sec) and spun at 200 rpm.

Removal of solute by blotting was carried out after the spin sequence. The entire product and blanket "sandwich" was squeezed six times between the compression rolls.

Frozen product weight was determined in a tared, insulated container immediately after the freezing exposure, after the spin sequence, the wash/spin sequence, and after the blotting treatment. Product temperature was measured by agitation of the frozen pieces in an insulated

Table 1–Predicted example concentrations for staged freezing and washing.

Solute	Total no. of stages	Stage no.	Solute conc (wt %)	Freezing temp (°F)
NaCl	1	1	23	−6.0
	2	1	23	−6.0
		2	11.5	17.7
	4	1	23.0	−6.0
		2	17.2	7.6
		3	11.5	17.7
		4	5.7	26.0
$CaCl_2$	1	1	29.5	−60.0
	2	1	29.5	−60.0
		2	14.7	14.0
	4	1	29.5	−60.0
		2	22.1	−8.2
		3	14.7	14.0
		4	7.4	25.4
Glycerine	1	1	67.0	−60.5
	2	1	67.0	−60.5
		2	33.5	11.0
	4	1	67.0	−60.5
		2	50.2	−10.0
		3	33.5	11.0
		4	16.8	25.5
Ethanol	1	1	76.3	−94
	2	1	76.3	−94
		2	38.1	−18
	4	1	76.3	−94
		2	57.2	−45
		3	38.1	−18
		4	19.1	14

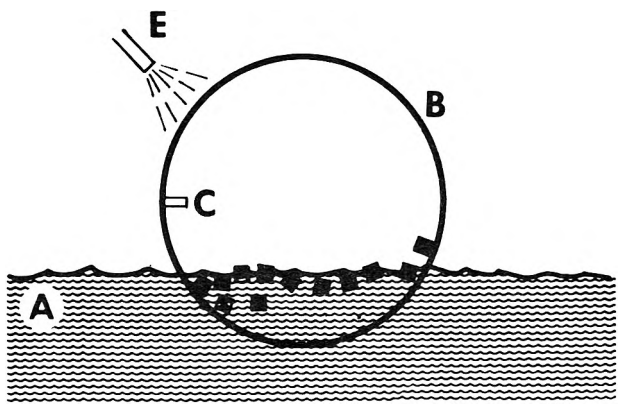

Fig. 4—Schematic of immersion freezing unit indicating heat transfer fluid A, rotating drum B, baffle C, product D, and freezant spray E. Counter-clockwise rotation.

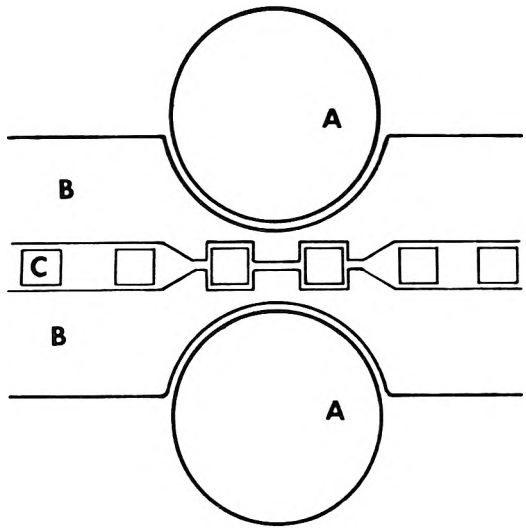

Fig. 5—Schematic or product blotting apparatus indicating compression rolls A, foam blankets B, and wet product C.

container containing a temperature probe. Experiments were repeated at least once.

The product from each run was stored in the frozen state at 0°F. Product from duplicate runs was combined. Subsamples of 10g were drawn from each well-mixed composite, combined with 90 ml of water and homogenized for 15 min in a 500 ml blender. The homogenized sample was filtered (White Ribbon #589) and analyzed for chloride ion by a coulometric silver titration procedure (AMINCO chloride titrator). Solute content was calculated by applying appropriate stoichiometric ratios. NaCl percentages are reported on a frozen product basis as weight of sodium chloride per weight of product.

To assess the effect of cooking, the frozen product (4 parts) was simmered in boiling water (1 part) for 8.0 min. The cooked product was drained, sampled (10g) and prepared for chloride analysis as above. Solute percentages were reported as weight of sodium chloride per weight of cooked, drained product.

RESULTS & DISCUSSION

Heat transfer characterization

Average product temperatures were determined after agitation in AF 23-0 at −2°F to 2°F for different time periods.

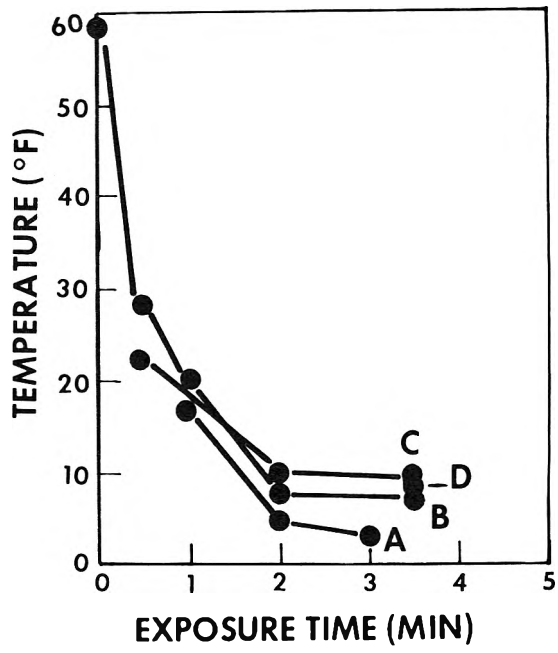

Fig. 6—Bulk product temperature of diced carrots A, garden peas B, snow peas C and green beans D.

These are shown in Figure 6. The time required to precool and freeze 90% of product water, the time elapsed to reach 20°F (Hayakawa, 1973), was less than or equal to 1.0 min for all products. Cooling of the products from 20°F to 10°F required an additional 0.5–1.0 min. Cooling to lower temperatures was very slow due to the low temperature driving force at the product surface (less than 10°F compared to 60°F at the initiation of freezing). These data do not distinguish a precooling period, a rapid phase change period, and a slow phase change period typical of air blast vegetable freezing.

Surface heat-transfer coefficients were estimated by applying Planck's approximate formula and by assuming the length of the phase change to be between 1.0–2.0 min. Surface heat transfer coefficients (h_s) of 50–150 BTU/hr°F ft^2 were calculated using this procedure. Mott (1964) reported h_s values of about 8 BTU/hr°F ft^2 for air at 2000 fpm and 30–50 BTU/hr°F ft^2 for $CaCl_2$ solutions.

Product temperature rose during countercurrent washing in the warmer wash solutions. Table 2 shows the temperature rise for contact with solutions at the indicated temperature and compositions. Temperature rise during washing can be maintained at 4–5°F by establishing and maintaining the wash liquor temperature at or near the ice formation temperature of the liquor (see data for peas).

Product temperature rose 3°F during the drain, spin sequence (15 sec drain and 30 sec spin) since the ambient air was not refrigerated. An additional warming of 1.8°F occurred during blotting experiments. These temperature changes were subtracted from the measured values to yield the temperatures reported in Table 2. Neither type of warming described here would be expected to occur in a fully insulated continuous process.

Mass-transfer characterization

Product weight and solute content were examined after prefreeze, freeze, and postfreeze processes. Results of these tests are shown in Table 3 (bulk weight changes) and Table 4 (solute content changes).

Two prefreeze blanching-cooling methods were tested (data

not shown). Steam-blanched and air-cooled carrot dice gained 33% more solute than steam-blanched and water-cooled carrot dice when compared after freezing. Additionally, the air-cooled carrot dice exceeded water-cooled product in solute content by 16% when compared after postfreeze blotting. High solute uptake by steam blanched and air-cooled product indicates that an alternate method (such as individual quick blanching, IQB) capable of maintaining the moisture balance (Bomben et al., 1973, 1975) would be desirable. All subsequent experiments employed water cooling of steam-blanched products.

A short centrifugal treatment was applied to each product shown in Tables 3 and 4. This treatment, a 15 sec drain and 30 sec spin at 200 rpm immediately following the freeze or wash treatment, reduced loosely adhering surface freezant. When applied to diced carrots, this mechanical "dewatering" effected a 22% reduction of bulk weight and a 21% reduction of product solute (data not shown). This large reduction reflects the large amount of freezant which adheres to the product immediately after its removal from the freezant solution. This retention is influenced by slow drainage due to the high viscosity of the freezant and the shape and dense packing of the carrot dices. Longer spin times did not effect greater reductions.

The bulk weight history of each product followed a similar pattern. Exceptions were attributable to the combined effects on drainage of piece geometry, piece size and solution viscosity. For instance, each product gained weight based on original fresh weight (2.8–8.7%) after cooling in water, and maintained this gain after freeze immersion (4.7–8.5%) and washing (2.8–8.5%). However, the net weight gain was reduced by blotting (0.0–3.7%). A net loss of bulk weight relative to raw product weight was not observed for any product frozen by this method.

The solute history for each product (as shown in Table 4) can be used to rank the effectiveness of each post-freeze treatment. The blotting treatment and washing treatment are of approximately equal effectiveness since they yield comparable reductions in solute levels. The respective reductions were 23% and 23% for beans, 12% and 25% for peas, and 61% and 56% for snow peas. Washing reduced the solute for carrots by 26%. Combined washing and blotting of product after freezing showed greatest reduction of solute from the frozen levels. Solute reductions were 37% for peas, 45% for carrots, 48% for beans and 76% for snow peas.

As indicated in the introduction, a 50% reduction of solute associated with surface-adhering liquor was anticipated for the wash treatment. However, smaller reductions of 23%, 25% and 26% were obtained for beans, peas and carrots. This result indicates that the surface contribution for these products amounts to approximately half of the total solute associated with the product. Deviation from the theory could also be explained by a partial depletion of solute from the surface-adhering liquors by diffusion into the product during freezing.

Consequently, when surface liquor replacement or exchange occurs during washing, the concentration of the replaced solution is less than 23% NaCl and the solute reduction by washing is less than predicted. The 60% reduction of solute obtained by applying SCAF to snow peas closely compares to the predicted result. This result was a reflection of the importance of surface liquors on this product of high surface area. The order of increasing experimental surface-to-volume ratios was beans (12 $in.^2/in.^3$), peas (14 $in.^2/in.^3$), carrots (16 $in.^2/in.^3$), and snow peas (26 $in.^2/in.^3$).

The effectiveness of the blotting technique militates strongly for its use alone or in conjunction with a wash. However, questions of foam lifetime and microbiology will require larger scale testing with a continuous unit. Some experience with these materials has been accumulated with juice presses and potato surface driers.

Table 2–Temperature rise resulting from 10 sec exposure of frozen product to wash liquor

Product	Wash liquor conc (% NaCl)	Wash temp (°F)	Bulk temp rise[a] (°F)
Beans	11.5	28	9
Carrots	11.5	21	6
	0.0	36	9
Peas	11.5	18	4
Snow peas	11.5	26	9

[a] Corrected for temperature rise during drain/spin sequences

Table 3–Percentage bulk weight changes during processing treatment

Product	Normalized wt (%)[a]				
	Blanched and cooled	Frozen[b]	Blotted[b]	Washed[b]	Washed[b] and blotted
Beans	104.7	104.7	100.0	102.8	100.0
Carrots	104.5	108.5	–	108.5	100.0
Peas	102.8	104.7	101.9	103.7	100.0
Snow peas	108.7	105.3	103.7	105.2	100.0

[a] Weight after indicated treatment divided by weight of fresh X 100
[b] Weight after centrifuging treatment

Table 4–NaCl concentration changes after post-freeze treatment

Product	NaCl percentage[a]			
	Frozen[b]	Blotted[b]	Washed[b]	Washed[b] and blotted
Beans	0.95	0.73	0.73	0.49
Carrots	4.49	–	3.29	2.45
Peas	0.98	0.86	0.73	0.62
Snow peas	2.55	0.99	1.08	0.61

[a] Mass NaCl/mass of frozen product X 100
[b] Concentration after centrifuging treatment

Product evaluation

AF frozen product was compared with blast frozen product for utilization evaluation. Products were compared on an as-is basis for direct consumption by the consumer and mixed with other vegetables. No tests were conducted using these products as part of a prepared dinner, or associated with a preseasoned sauce even though these applications might be suitable for product containing high salt level (carrots).

Since product designed for direct consumption would require in-home cooking in boiling water, preliminary tests were conducted to evaluate the additional reductions of solute which might be achieved. Product was cooked in 1 part boiling water for each 4 parts vegetable (see Procedures). The solute reductions shown in Table 5 and 6, were 7% (beans), 12% (carrots), 18% (peas) and 50% (snow peas) when applied to product frozen without post-freeze treatments in addition to centrifuging. Cooking yielded solute reductions of 4% (beans),

Table 5—NaCl reduction after cooking of frozen product

Product	Frozen (% NaCl)[a]	Cooked (% NaCl)[a]	Reduction (%)
Beans	0.97	0.90	7
Carrots	4.52	3.97	12
Peas	1.01	0.83	18
Snow peas	2.58	1.29	50

[a] Mass NaCl/mass frozen product X 100

Table 6—NaCl reduction after cooking of frozen, washed, and blotted product

Product	Before cooking (% NaCl)[a]	After cooking (% NaCl)[a]	Reduction (%)
Beans	0.51	0.49	4
Carrots	2.48	2.19	12
Peas	0.65	0.48	26
Snow peas	0.64	0.57	11

[a] Mass NaCl/mass frozen product X 100

Table 7—Cost (¢/lb) comparison of aqueous immersion, air blast, and fluidized bed freezer

	Air blast		Fluid bed		Aqueous immersion	
Item	1974	1979	1974	1979	1974	1979
Power	0.14	0.35	0.17	0.43	0.11	0.28
Refrigerant	0.0	0.0	0.0	0.0	0.05	0.07
Fixed cost	0.24	0.34	0.17	0.24	0.11	0.16
Labor	0.28	0.39	0.20	0.28	0.20	0.28
Maintenance	0.03	0.04	0.03	0.04	0.03	0.04
Total no wt loss	0.69	1.12	0.57	0.99	0.50	0.83
Total for 1% wt loss	0.79	1.26	0.67	1.13	0.50	0.83
Total for 2.5% wt loss	0.94	1.47	0.82	1.34	0.50	0.83
Total for 5.0% wt loss	1.19	1.82	1.07	1.69	0.50	0.83

12% (carrots), 26% (peas) and 11% (snow peas) when applied to product frozen with post-freeze treatment. The difference in magnitude between the average reduction (13%) for cooking of post-freeze treated vegetables and the average reduction (22%) for untreated product probably reflects the relative amount of surface material available for removal.

As a point of comparison, the reader should note that the concentration of NaCl used in the liquid added to canned vegetables is typically about 1.5–2.0% (Joslyn and Timmons, 1967). Moreover, Handbook 8 (Watt and Merrill, 1963) estimates salt contents in canned vegetables at 0.6%. This handbook also indicates that peas commercially frozen in air have a salt content of about 0.3% which is a residual from the quality separation process applied to this product. Lima beans also receive this treatment and have comparable NaCl residues. The salt content of prepared soup is indicated at 2–2.5%.

Energy consumption

The only energy requirement which is equal in magnitude for both air-blast and aqueous freezing systems is the requirement represented by the sensible and latent heat load of the frozen product. Additionally, both systems require capacity to compensate for heat penetration into the equipment, but adequate insulation can minimize this contribution.

The principal energy requirement where numerical differences can be anticipated is the requirement for maintaining the transfer fluid flow volocity necessary for rapid freezing. The relative order of energy consumption via fluid movement can be calculated for a hypothetical freezer in which the transfer fluid flows through a monolayer of product (3/8 in. cubes). The energy consumed is proportional to the product of four factors: the cube of the linear fluid velocity, the fluid density, bed and particle shape factors, and the time for freezing (Generaux et al., 1973). Experimental data (Mott, 1964; Slavin, 1964) and the Planck equation, are available to facilitate this calculation.

Energy consumption for freezing in 3.3 min using an AF 23-0 at 0°F and 15 fpm (a velocity slightly greater than the natural convection value) was compared to energy consumption for freezing in 7.0 min using air at −30°F and 750 fpm [as indicated for a commercial average (Fennema, 1973)]. At this condition the energy required for movement of air required 210 times the energy required for the movement of an AF and is a significant percentage (34%) of the net product load. The size of the freezer utilizing AF is also smaller (53%) reflecting the difference in freezing times.

Since the energy utilized for fluid movement increases approximately as the velocity cubed, the advantage of utilizing an AF is even greater at higher air velocities. For instance, at an air velocity great enough to freeze the product in 3.3 min (2500 fpm), the energy consumed for air movement is approximately 10^4 times greater than that for freezing in an aqueous freezant.

Several points of energy consumption are unique to the AF freezing systems. One of these occurs as an additional heat load which must be removed before or during storage at 0°F. This load occurs as the product warms during the SCAF (when applied) and amounts to 10 BTU or 5% of the net sensible and latent heat requirement. Finally, the AF will have a limited life expectancy before disposal, and its disposal will also contribute to the system heat load. The lifetime of the AF will be limited by the microorganisms and leached solids which it contains. However, the potential of these contaminants for limiting the freezant lifetime is minimized by the nature of the freezant. The high salinity and low temperature of the freezant will preclude the growth of microbes of significance to high product quality and public health (Weiser et al., 1971). Furthermore, the low temperature of the freezant quickly solidifies the product surface so that little leaching can occur. Although no data are available to ascertain the AF lifetime, the additional energy load for evaporation of the freezant would be 4% of the net product load if each lb of freezant were able to process 100 lbs of product.

Economic estimate

Data reported earlier for air-blast and fluid-bed freezing (Rasmussen, 1967) were used with published economic indicators (Anon, 1975a) to calculate current cost for 1974. These data were also extrapolated to 1979 using the assumption of a 2.5 factor increase in electric power costs (Ludwig, 1975). Additional assumptions included: power costs for SCAF which were 20% less than power costs for air blast by reason of reduced fan power consumption (and allowing for freezant disposal); fixed costs for SCAF which were 53% less than for air blast by reason of reduced equipment size which results from higher freezing rate; labor and maintenance costs which

were equivalent to those for fluid-bed freezing, and cost of refrigerant solute (NaCl) which was 5 cents/lb (Anon, 1975b) assuming a 1% residue.

The calculated data are shown in Table 7. Totals are shown for factors mentioned above and for 1%, 2.5% and 5% weight reductions due to evaporation during the freezing process. If weight loss factors are excluded, the cost of freezing (1974) is least for SCAF with fluid bed and air-blast freezing being progressively more. With escalation of energy costs, the favorable position of SCAF improves, yielding a cost which is 16% less than fluid bed and 26% less than air blast freezing.

Moreover, if the weight loss of product is accounted as a freezing cost, the cost comparison strongly favors the use of SCAF. At a 2.5% weight loss, product selling for 10 cents in 1974 (or 14 cents in 1979), the costs per lb are AF 0.50 cents (0.83 cents), fluid bed 0.82 cents (1.34 cents), and air blast, 0.94 cents (1.47 cents).

CONCLUSIONS

1. Vegetable pieces frozen in 23% NaCl (AF 23-0) aqueous solutions and given suitable post-freeze treatments reach solute levels ranging from as low as 0.48% NaCl for peas to 2.19% for diced carrots. Lowest salt levels are comparable to levels of salt in canned products and highest values are comparable to levels in remanufactured items such as soup.

2. Solute reduction methods of blotting or washing in 11.5% NaCl produce comparable reductions of solute, and combined washing and blotting was more effective than either method alone.

3. Freezing times are short. Vegetable pieces can be cooled and frozen to 20°F in aqueous freezant (NaCl) in 1.0 min or less.

4. Estimated processing cost of AF frozen product is less than cost of air blast or fluidized bed frozen product. This advantage is increased when weight losses are included in the calculation.

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Ms received 9/13/75; revised 11/23/75; accepted 12/2/75.

Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others which may also be suitable.

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EXTRUDED FRENCH FRIES FROM DEHYDRATED POTATO GRANULES PROCESSED BY A FREEZE-THAW TECHNIQUE

ABSTRACT

Some physicochemical properties of dehydrated potato granules were studied in relation to their suitability for extruded French fries. The freeze-thaw granules exhibited higher water-binding capacity (WBC), lower bulk density and larger particle size than the add-back granules. Microscopic examination revealed that relatively rapid and complete rehydration was associated only with the freeze-thaw granules. The add-back granules indicated a thin membrane of insoluble, completely retrograded amylose that was revealed immediately on contact with water while the interior granule absorbed water slowly. These differences were attributed to the respective processing techniques. Trials indicated that the ratio of 1:2.6 rather than 1:2 of granules to water (w/v) is superior for reconstitution and French fry extrusion of the freeze-thaw granules. Production of good quality extruded French fries from these granules is possible with the use of a mixture of binders such as guar gum, stabilized high amylose corn starch, crosslinked pregelatinized corn starch, and hydroxypropylmethylcellulose.

INTRODUCTION

A NEW METHOD for the production of dehydrated potato granules by a straight through freeze-thaw process without add-back (re-circulation) of a large proportion of the product has been described by Ooraikul (1973). This type of granule has been found superior in nutritional quality (Jadhav et al., 1975) and in flavor, color and texture (Ooraikul, 1973) to that from the commercial add-back process. Preliminary work has shown that the use of good quality granules improves the flavor of an extruded product for French fried potatoes. Fabricated French fries made with a French fry potato mix have a number of advantages including dry storage of the mix, 90-sec frying time, reduced shrinkage, up to 2-hr holding time after frying, and year-round uniformity of product. In recent years, the demand for such product has increased.

Since there are differences between the freeze-thaw granules and those from the commercial add-back process, the suitability of the freeze-thaw granules for extruded French fries has been examined. Also water-binding capacity was studied to provide a method to distinguish freeze-thaw from add-back potato granules.

MATERIALS & METHODS

Tubers

Cultivar Netted Gems grown in southern Alberta were used with a sp gr range of 1.080–1.090.

Dehydration processes

Dehydrated potato granules were processed by the straight through freeze-thaw process developed by Ooraikul (1973). Samples of commercial granules and French fry mix were obtained from a local add-back processing plant. The following steps were involved in the production of dehydrated potato granules.

Add-back process. Peeling, trimming, slicing, washing, precooking-water blanching, water cooling, steam cooking, mashing and mixing with dry granules and additives, conditioning or tempering, remixing, air lift drying, cyclone granule trapping, fluidized bed drying, fluidized bed cooling and sieving.

Straight through freeze-thaw process. Peeling, trimming, slicing, and washing as above; steam cooking, freezing, thawing, pre-drying in presence of additives, granulation, final drying, cooling, and sieving. Hot mashing of cooked potatoes as used by Ooraikul et al. (1974) was eliminated. Mashing of the thawed product was achieved in the pre-drying step. Both the processes are described in detail elsewhere (Ooraikul et al., 1974; Ooraikul and Hadziyev, 1974; Jadhav et al., 1975).

Bulk density

The bulk density of the granules was measured by filling the granules into a 250 ml graduated cylinder up to the 200 ml mark while gently tapping the cylinder against the floor until there was no further packing of the granules. The 200 ml granules were then weighed and the bulk density was calculated in g/cc.

Water-binding capacity

The percent of bound water of the dehydrated potato granules as determined by the method of Medcalf and Giles (1965) with the modification suggested by Morrow and Lorenz (1974) was used as the water-binding capacity (WBC). The potato granules, in amount of 0.5g, were added to 10 ml distilled water in a tared 15 ml centrifuge tube. The tubes were stoppered and agitated for 1 hr with a wrist-action shaker. This was followed by centrifuging for 10 min at 2,200 × G. Excess water was allowed to drain off for 10 min. The tubes were weighed to determine the bound water which was reported as an average of four determinations.

Extruded French fries

A 50-g batch of freeze-thaw granules containing added binders was reconstituted in water with gentle stirring. After 10 min, the dough was extruded through a cookie press modified to produce strips with a cross-section 1 cm^2. The extrusion head was made of 1 cm thick Teflon sheet in which was cut a tapered hole 1.5 cm^2 on the inside and 1.0 cm^2 on the outside; this shape was necessary to avoid ragged edges of the extruded French fries. The freeze-thaw French fry mix contained 0.75g Textaid, a crosslinked pregelatinized corn starch; 0.25g Crisp Film, a stabilized high amylose corn starch (National Starch and Chem. Co. Canada Ltd., Toronto, Ont.); 0.75g guar gum, a galactomannan (General Mill Chem. Inc., Minneapolis, Minn.); and 0.50g sodium carboxymethylcellulose (CMC) of 0.7 degree of substitution and high viscosity (Hercules Inc., Wilmington, Del.) or Methocel 90HG, hydroxypropylmethylcellulose (Dow Chem. Co., Sarnia, Ont.) per 50g of the mixture.

For French fries made from add-back granules, the commercial add-back mix was used. The normal commercial method was followed for reconstitution of adding 2 vol of water to a unit weight of the add-back mix. All extruded strips were fried in a shortening (Canada Packers Ltd.) containing a standard mixture of antioxidant and monoglyceride citrate as an anti-spattering agent, for 90 sec at 185°C and immediately evaluated for texture, flavor and appearance by a preference test (ranking difference analysis) as outlined by Larmond (1970). A batch of the freeze-thaw fries (I) and duplicate batches of the add-back fries were then displayed for the triangle test (Larmond, 1970). A panel of 17 uninformed judges were asked to evaluate the fries for texture and flavor and identify the odd sample. The oil content of the fries was determined immediately after frying. The fries were dried in vacuum (80°C and 68 cm Hg) to a constant weight. The dry solid was then extracted in Soxhlet using petroleum ether (b.p. 40–60°C) as a solvent.

RESULTS & DISCUSSION

THE RESULTS on the WBC of dehydrated potato granules obtained by the freeze-thaw and add-back processes are presented in Table 1. It is interesting to note that the average WBC of the freeze-thaw granule mixture was significantly

higher ($P < 0.05$) than that of the add-back mixture. When the whole mixture was passed through a series of sieves, the particle size distribution for the add-back granules was quite uniform with nearly 82% of the granules below the 100 mesh sieve-size. However, the freeze-thaw granules showed a wider range of particle size, namely 60, 80 and 100 mesh were present as 40, 36 and 20% respectively. The WBC values recorded for both types of granules were influenced by their size and bulk density. As the granule size increased, the total intergranular space increased and consequently the packed volume of the granules enabled more water to be retained in the intergranular space. The increase in WBC of potato starch was also considered by Morrow and Lorenz (1974) to be governed solely by the starch grain size. High bulk density is generally associated with the finest size of granules such as the add-back type. Cooley et al. (1954) noted a decrease in bulk density of granules as a result of a rapid rate of drying in granule processing step. This lower bulk density of the freeze-thaw granules may be caused by the shorter drying period than that used for the add-back granules.

The freeze-thaw granules absorbed water more rapidly than the add-back granules when added slowly to water and stirred in order to make a dough. Microscopic examination indicated that presence of a thin membrane with the rehydrated add-back granules but not with the rehydrated freeze-thaw granules (Fig. 1). This membrane, composed of insoluble, completely retrograded amylose starch fraction, was revealed immediately on contact with water while the interior granule absorbed water slowly. The compositional identity of the membrane was based on its negative response to iodide-iodine and hydroxylamine-ferric chloride tests for starch and pectin as recommended by Johansen (1940) and Reeve (1959), respectively. The membrane may affect the rehydration rate by protecting the granule from immediate contact with water. This may explain the longer rehydration rate of the add-back granules. The higher WBC of the freeze-thaw granules is also probably partly a result of the difference in their particle surface area from that of the add-back granules. The differences in the particle size of swollen granules are illustrated in Figure 1. A similar study on WBC of potato starch indicated its dependency on the final size of the starch grain in dilute suspension (Morrow and Lorenz, 1974). Reeve (1954) studied histological changes in various forms of dehydrated potato products and found that gelled starch in cells of potato granules did not swell sufficiently to completely fill the cells, leaving small spaces in the angles formed by the cell walls. He produced these granules in laboratory-scale operation by dehydration of cooked and whipped potatoes in the presence of added dry potato powder. The shape of dehydrated or rehydrated granules is another factor that should influence the WBC since it is related to the total surface area of the granule. As shown in Figure 1, the freeze-thaw granules exhibited irregular or angular shapes while the add-back granules were more or less round and smooth.

Production of the freeze-thaw granules involved freezing and thawing of cooked potatoes before dehydration. It is known that ice crystals formed by freezing create a more porous starch grain within the porous potato granules to facilitate rapid drying of the cooked potato mash. Reeve (1954) reported that frozen cooked potatoes have a porous structure of the granules when dried, and that freezing and thawing alter the moisture-reabsorbing capacity of the starch gelatinized grain. Greene et al. (1948) also observed great alterations in the macrostructure of the cooked potatoes by freezing and thawing.

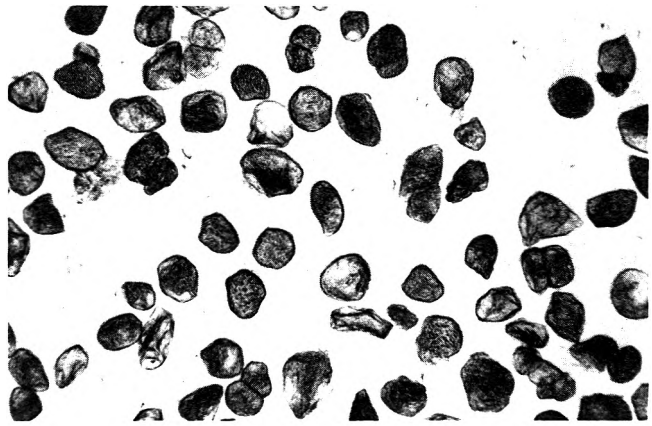
The effect of time and temperature during final drying on the WBC of the freeze-thaw granules is illustrated in Table 2. The granules processed for a longer period of time at the same temperature (93°C) showed a tendency to reabsorb less water than those at normal processing conditions because of impair-

Table 1—The relationship between water-binding capacity, size and bulk density of two types of dehydrated potato granules

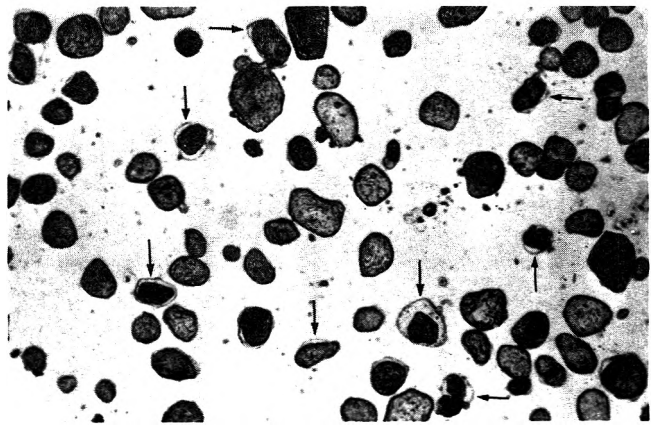
	Freeze-thaw granules[a]			Add-back granules[a]		
Fisher sieve size mesh (/in)	% of Total	Bulk density g/cc	WBC[b] %	% of Total	Bulk density g/cc	WBC[b] %
Whole mixture	100	0.726	394	100	0.945	284
+60	4	–	421	0	–	–
–60	40	0.705	402	5	–	379
–80	36	0.758	375	13	0.846	340
–100	20	0.786	348	82	0.952	272

[a] Moisture content: Freeze-thaw granules, 5.4%; Add-back granules, 7.2%.

[b] Water-binding capacity (% bound water); Least significant difference ($p < 0.05$): 16 for WBC of whole mixture; 19 for WBC of the freeze-thaw granules; 12 for WBC of the add-back granules.



FREEZE-THAW



ADD-BACK

Fig. 1—Freeze-thaw and add-back granules below the 100 mesh sieve-size (X 41.5 magnification) suspended in water at 22° C showing presence of membrane in latter indicated by arrows. Average granule size: freeze-thaw, 0.189 mm; add-back 0.165 mm, least significant difference ($p < 0.05$) = 0.013 mm.

Table 2—The relationship of water-binding capacity to processing conditions for freeze-thaw dehydrated potato granules

Fisher sieve size mesh (/in)	% WBC[a] of the freeze-thaw granules		
	Normal drying[b]	Longer drying[c]	LSD ($p < 0.05$)
+60	421	390	19
−60	402	373	13
−80	375	350	20
−100	348	326	15

[a] Water-binding capacity (% bound water)
[b] Final granule drying stopped when exhaust air temperature reached 60° C within 5–10 min. Drying air temperature was 93° C. The moisture content of the product was 5.4%.
[c] Final granule drying continued until the exhaust air temperature was 80° C. Total drying time was 20–25 min using dry air at 93° C. The moisture content of the product was 4.1%.

Table 3—Effect of increasing amounts of water on rehydration and extrusion characteristics of French fry mix made with freeze-thaw granules

Granule/Water ratio (w/v)	Rehydration characteristics		Extrusion characteristics
	Immediately	After 10 min	Immediately
1:2.0	very dry, powdery, uneven	very dry, crumbly to powdery	very ragged edges, fragile, much breakage
1:2.2	dry, crumbly, uneven	dry, crumbly	very ragged edges, fragile, much breakage
1:2.4	still dry, less crumbly	dry, slightly moist	ragged edges less severe, less breakage
1:2.5	moist, very good	moist, somewhat cohesive yet not gluey	very good very smooth edges
1:2.6	moist, more cohesive	moist, cohesive yet not gluey or pasty	very good very smooth edges

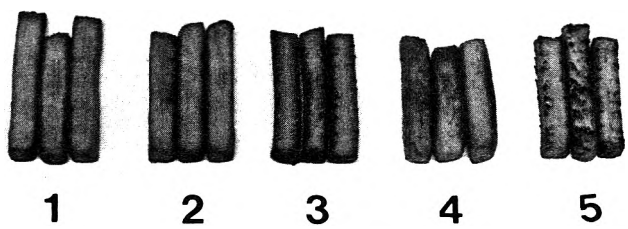


Fig. 2—The effect of different water reconstitution ratios on the appearance of French fries made from add-back (1) and the freeze-thaw French fry mix (2–5). The whole granule mixture as indicated in Table 1 was used in the mix. 1:2 reconstitution ratio (w/v) for 1, 3 and 5 while 1:2.6 for 2 and 4. Methocel 90HG (2,3) or CMC (4,5) was used as one of the binders. Note ragged edges in 3 and 5 and round shapes in 4.

ment to the granule structure. In the add-back process, there is no freeze-thaw step. Instead, the retrogradation of a free starch is achieved by conditioning or tempering under a stream of cold air. The conditioned moist potato mix also showed an inadequate porosity of the starch grain. As a result, the final drying of the mix required a relatively high time-temperature combination (100–200°C for 10–30 min). It therefore appears that the extent of rehydration or water-binding capacity of dehydrated potato granules is affected by the processing technique.

The WBC and rehydration tests indicated that the freeze-thaw granules require more water for reconstitution than the add-back granules; this is of importance when dough is extruded for French fries. The 1:2 proportion of granules to water (w/v) used for add-back granules is not suitable for freeze-thaw granules; Table 3 shows that the dough became moist and somewhat cohesive at the ratio of 1:2.5. By further increasing the amount of water added it was found that the 1:2.6 ratio was more suitable for rehydration and extrusion of the freeze-thaw granules than the 1:2 ratio. The use of inadequate amounts of water led to a dough that was too dry and crumbly resulting (Fig. 2) in extruded fries having a ragged appearance and shape and an unacceptable taste because of poor texture. A similar problem was encountered by Jericevic and LeMaguer (1975). They reported that the initial moisture content of the granules is the critical factor and that it should be maintained at optimum level to decrease the rate of rehydration and allow uniform rehydration and mixing of the granules with water. As an alternative, they recommended that the existing ratio of 1:2, granules to water (w/v), for making French fry dough should be corrected. The modified procedure of reconstitution which utilized nearly 30% more water than that used for the add-back granules is in keeping with the percent increase in the WBC which is approximately 33%.

Preliminary experiments, using 1:2.6 reconstitution ratio for the freeze-thaw granule mix showed that CMC or hydroxypropylmethylcellulose (Methocel 90HG) alone is unsuitable for making extruded French fries. The extruded strips were soft in texture which showed a lack of adhesion. The added water evaporated from the surface very quickly in a boiling action causing the fries to blow apart during frying. Also oil absorption of the fragmented fries was extremely high. These results suggested that composition and properties of binders used in the French fry mix are very cirtical for the success of extruded French fries. The problem of explosion and extremely high oil absorption was eliminated by using a formulation consisting of 1.5% guar gum, 0.5% stabilized high amylose corn starch (Crisp Film), 1.5% crosslinked pregelatinized corn starch (Textaid) and 1% CMC or Methocel 90HG on w/w basis of granules for the freeze-thaw French fry mix. The addition of guar gum, a galactomannan, to the granules offered several processing advantages such as rapidly absorbing and tying up free water during reconstitution, preventing separation and migration of the free water while frying and allowing the product to retain moisture longer. Because a linear polymer has a better film and fiber strength than a branch polymer the use of high amylose starches of Crisp Film type have been recommended in both supported coatings and unsupported films of certain foods (Hullinger et al., 1973). Also the high amylose starches and their derivatives have found increasing acceptance in par fried potatoes because of their ability to retard oil penetration and impart crisp and crunchy coating with better color. The cross-linked pregelatinized corn starch was particularly important in development of natural solid texture and its subsequent retention under the frying conditions of the extruded French fries. In Table 4 is shown an evaluation based on oil content, texture, flavor and appearance of the extruded French fries. The freeze-thaw fries (I) were superior in relation to flavor and texture when compared to the fries made from the commercial add-back French fry mix. Also, the triangle

Table 4—Comparison of oil content, texture, flavor and appearance of extruded French fries

Type of extruded French fry[b]	Moisture %	Oil/fried sample %	Ranking difference analysis score[a]			Overall Preference[c]
			Texture	Flavor	Appearance	
Add-back	49.6	7.0	−0.56a	−0.78a	0.35a	2nd
Freeze-thaw I	49.3	9.7	0.28b	0.36b	0.28a	1st
Freeze-thaw II	44.4	18.1	0.28b	0.43b	−0.63b	3rd

[a] Samples ranked on the basis of 1st, 2nd and 3rd preference and assigned scores of 0.85, 0 and −0.85, respectively (Larmond, 1970); means of 12 judgements followed vertically by the same letter are not significantly different ($p < 0.05$).
[b] Reconstitution ratio (w/v), 1:2 for the add-back and 1:2.6 for the freeze-thaw granule mix; the freeze-thaw fries contained Methocel 90HG (I) or CMC (II) as one of the binders; finish frying for 90 sec at 185°C.
[c] The freeze-thaw fries (II) were given the last preference on the basis of abnormal shape and high oil content.

test showed that the freeze-thaw fries (I) were organoleptically distinguishable from those made of the add-back French fry mix, as 14 out of the 17 panelists were able to correctly identify the odd sample (10 correct answers would be necessary to establish significance). The overall quality of fries (II) that contained CMC as one of the binders was affected by high oil absorption. Moreover, these fries showed a tendency to have rounded edges (Fig. 2). Oil absorption by the extruded fries and their shape and appearance can be controlled with the use of Methocel 90HG binder (Table 4, Fig. 2). Methocel products are known to form oil-resistant thermal films at relatively high temperatures that are less prone to rupture due to migration of water from potato strips during frying and reduce oil absorption (Glicksman, 1969).

In conclusion, the production of good quality extruded French fries from the freeze-thaw granules is possible with the use of appropriate binders and subsequent rehydration (w/v) in 2.6 volumes of water.

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Ms received 10/14/75; revised 2/6/76; accepted 2/8/76.

The authors gratefully acknowledge the financial support of the Alberta Potato Commission.

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VITAMINS A AND C IN RIPE TOMATOES AS AFFECTED BY STAGE OF RIPENESS AT HARVEST AND BY SUPPLEMENTARY ETHYLENE

ABSTRACT

The ascorbic acid (vitamin C) and β-carotene (provitamin A) contents of ripe tomatoes harvested at different stages of ripeness or treated with supplementary ethylene were determined. Vitamin C activity in 100g ranged from 15–50% U.S. Recommended Daily Allowance (RDA) among cultivars. Vitamin C activity was not affected by stage of ripeness at harvest, but was slightly higher in a few cultivars treated with ethylene. The vitamin A activity in 100g ranged from 10–140% U.S. RDA among cultivars. Vitamin A activity was not affected by ethylene, but was slightly higher in ripe fruit that had been harvested ripe than those harvested mature-green. However, fruit harvested mature-green or breaker, the stages at which most fresh market tomatoes are harvested, did not differ in vitamin A activity for three of the four cultivars tested.

INTRODUCTION

A FRESH, ripe, tomato fruit weighing 100g supplies 10.4–44.6 mg ascorbic acid (vitamin C) and 0.21–0.80 mg β-carotene (provitamin A) (Lincoln et al., 1943). Vitamin contents of tomatoes differ with cultural practices, cultivars and postharvest handling practices (Hamner and Maynard, 1942).

Tests on the effect of stage of ripeness at harvest and of supplementary ethylene on ascorbic acid content have produced contradictory results. Clow and Marlatt (1930) observed that ripe tomatoes harvested as mature-green and as ripe fruit contained the same quantity of ascorbic acid. In contrast Scott and Kramer (1949) and Pantos and Markakis (1973) reported that ripe fruit harvested mature-green had ascorbic acid levels different from those harvested ripe. Clow and Marlatt (1930) found that the ascorbic acid contents of tomatoes treated with and without ethylene were similar; whereas, Jones and Nelson (1930) found that ethylene-treated tomatoes contained less ascorbic acid than those untreated.

Ascorbic acid content increases and then decreases with ripening (Malewski and Markakis, 1971), thus conflicting results could have been due to differences in ripeness at time of analysis. Some workers could have considered the fruit ripe when the ascorbic acid level happened to be maximum; whereas, others could have considered the fruit ripe at a later stage when the level was decreasing. These workers determined ripeness visually, so the stage at which ascorbic acid was analyzed is not known.

Earlier studies indicate that the stage of ripeness at harvest or supplementary ethylene has no effect on the vitamin A potency of the fruit (Jones and Nelson, 1930; House et al., 1929; and Morgan and Smith, 1928). Potency was based on the survival of rats that were fed canned juice of canned fruit prepared from the treated lots.

We reexamined the effect of stage of ripeness at harvest and of supplementary ethylene on ascorbic acid and β-carotene of ripe tomatoes, determining ripeness objectively by the light-transmittance technique (Worthington, 1974). Fresh market tomatoes generally are harvested prior to or at incipient ripening; thus, fruit at these stages, as well as ripe fruit, were used for the study on stage of harvest. Green fruit prior to ripening are called "mature-green" and those at incipient ripening are termed "breaker" (USDA, AMS, 1975). Supplementary ethylene is used to hasten ripening of many mature-greens, but not breakers; thus only mature-greens were used for the ethylene study.

MATERIALS & METHODS

Stage of harvest

Tomato fruit of ten cultivars were harvested when mature-green and ripe in 1973, and five cultivars were harvested at the mature-green, breaker and ripe stages in 1974. Two lots, each of ten fruit, of each maturity were selected in 1973, and two to five lots of each maturity were selected in 1974. Fruit were held at 21.1°C and analyzed when ripe. The conclusions of the studies for the 2 yr were similar; thus most of the comments are based on the 1973 study.

Ripeness was determined objectively by the light-transmittance technique as described by Worthington (1974) and is expressed as the difference between the absorbance (ΔA) at two wavelengths (nm). The mature-green fruit had a ΔA (510–600 nm) of 0.2–0.4 and required about 5 days at 21.1°C to ripen to breaker stage, which had a ΔA (510–600 nm) of about 1.5. Ripe fruit had a ΔA (600–690 nm) of 2.6–3.0. An exception to this was the orange 'Caro-Red' fruit which had a ΔA (600–690 nm) of 0.6–1.0. Fruit were rated ripe on the basis of comments by a sensory panel in earlier tests.

Ethylene

'Walter' tomatoes were obtained from 11 Florida growers–four in May, three in December, and four in March. A box of graded fruit from each grower was placed in a commercial gassing chamber, where the ethylene concentration was calculated to be 8000 ppm, for 24–36 hr at 22.2°C. A second box was held at a comparable temperature during the treatment period, and both boxes were shipped to Beltsville, Md., where the fruit were ripened at 21.1°C. Two lots of 15 fruit each were analyzed from each box for ascorbic acid and β-carotene when the ΔA (600–690 nm) was 2.6–3.0.

Ascorbic acid

The ascorbic acid was analyzed by a modified AOAC method (AOAC, 1970). A 7-mm wedge from each of the 10 or 15 fruit samples was immersed directly into the meta-phosphoric-acetic acid extraction solution and macerated. The macerate was centrifuged, and 2,6-dichloro-indophenol Na salt used in the titration was obtained from Sigma Chemical. Each sample was analyzed in duplicate. The vitamin C is reported in mg per 100g fresh weight (gfw) and as percentage of U.S. Recommended Daily Allowance (RDA), which is 60 mg. The percentages are expressed in multiples of five for levels from 10 through 50% and in multiples of ten for levels above 50% (USDHEW, 1973).

β-Carotene

A 7-mm wedge from each of the 10 or 15 fruit samples was heated to 74°C in a microwave oven and stored at −20°C until analysis. The β-carotene was analyzed by a modified AOAC method (AOAC, 1970) and was extracted with an acetone-hexane mixture. The MgO-Hyflo-Supercel chromatographic column was washed with 0.5% acetone in hexane so that isomerization of carotenoids would be minimized (Wiseman et al., 1952). β-Carotene was eluted with 5% acetone in hexane. The concentration of β-carotene was based on the absorbance at 451 nm with an extinction coefficient of 2505 (Goodwin, 1955). Vitamin A

activity is presented as percentage of U.S. RDA. One International Unit (IU) of vitamin A equals 0.6μg β-carotene and 5000 IU equals 100% U.S. RDA. Percentages are expressed in multiples of five or ten as described for ascorbic acid (USDHEW, 1973).

RESULTS & DISCUSSION

Stage of harvest

Average ascorbic acid contents of ripe tomatoes were 17.6 and 18.7 mg/100 gfw, respectively, for fruit harvested mature-green and ripe (Table 1). These values were not significantly different and represented 30% U.S. RDA of vitamin C. Of the cultivars, only 'Double-Rich' had significantly more vitamin C in fruit that had been harvested ripe than those harvested mature-green. The U.S. RDA of vitamin C was 5 percentage points more in fruit harvested ripe than that harvested mature-green.

The ascorbic acid contents ranged from 13.7–31.8 mg/100 gfw for the cultivars harvested ripe (Table 1). A 100-g portion of 'Double-Rich' contained 31.8 mg ascorbic acid, which was 50% U.S. RDA. 'Heinz 1350,' a processing cultivar, contained 23.6 mg/100 gfw, which was 74% of the value for 'Double-Rich.' The ascorbic acid in 100g of the remaining cultivars ranged from 13.7–19.4 mg, which were 44 to 61% of the value for 'Double-Rich.' These quantities represented 20–30% U.S. RDA of vitamin C.

The differences in ascorbic acid content were greater among cultivars than between ripe fruit harvested as mature-green or ripe. The largest difference in U.S. RDA was 30 percentage points among cultivars, and only 5 percentage points between stages of maturity at harvest. In 1974, the differences also were larger among cultivars than among harvest stages, which included mature-green, breaker and ripe.

The average β-carotene contents of tomatoes ripened on and off the plant were 475 and 385 μg/100 gfw, respectively, which were significantly different (Table 2). The average values did not include 'Caro-Red,' which was developed for high β-carotene content. In terms of vitamin A activity, 475 and 385 μg are equivalent to 15% U.S. RDA.

The β-carotene level of fruit ripened on and off the plant differed significantly only with some of the cultivars (Table 2). Fruit of 'Fantastic,' 'Double-Rich,' 'Heinz 1350,' one set of 'Walter,' and 'Caro-Red' contained higher β-carotene when ripened on the plant. Except for 'Caro-Red,' the difference in the U.S. RDA of vitamin A due to harvest maturity was only 5 percentage points. The difference was 80 percentage points for 'Caro-Red.' Although the β-carotene levels of the remaining cultivars were not significantly different, the levels were higher in fruit ripened on the plant.

The second year's study confirmed that the β-carotene contents of ripe tomatoes varied directly with ripeness of the fruit at harvest (Table 3). The average content of a 100g ripe fruit was 82 μg greater when harvested as ripe than as breaker and 103 μg greater when harvested as breaker than as mature-green. Thus, although the increases were not always significant, β-carotene of all cultivars increased with ripeness of fruit at harvest. McCollum (1954) showed that tomato fruit exposed to sunlight during ripening contained more β-carotene than those ripened in shade. In our study, fruit harvested at a riper stage were exposed to more sunlight, which probably was the cause for the difference.

The differences in vitamin A activity were greater among cultivars than between stage of ripeness at harvest (Table 2). The vitamin A activity of 'Caro-Red,' the cultivar with highest activity, was 5–11 times that of other cultivars. The difference between stage of maturity at harvest was only 1/4 or less of the differences observed with cultivars.

Ethylene

The average ascorbic acid content of tomatoes treated with supplementary ethylene was higher than that of untreated fruit, and the differences were significant for May and December lots (Table 4). However, differences may not have been due to the direct effect of ethylene on ascorbic acid. Ascorbic acid in tomatoes increases to a maximum level and then decreases with ripening (Malewski and Markakis, 1971). The supplementary ethylene may have hastened color development sufficiently for the ripe color to develop when the ascorbic acid was near the maximum level; whereas, the untreated fruit may have developed ripe color when ascorbic acid was decreasing. In terms of percentage of U.S. RDA of vitamin C, only the

Table 1–Ascorbic acid content and percent of U.S. Recommended Daily Allowance (RDA) of vitamin C in ripe tomatoes of several cultivars that were harvested mature-green or ripe in 1973[a]

	Stage of harvest			
	Mature-green		Ripe	
Cultivar	mg/100 gfw	% US RDA[c]	mg/100 gfw	% US RDA
Double Rich	26.6 b[d]	45	31.8 a	50
Heinz 1350	22.8 bcd	40	23.6 bc	40
Fantastic	18.5 defg	30	19.4 cde	30
Manapal	17.2 efgh	30	18.9 def	30
Rutgers	15.8 efgh	25	18.8 def	30
Cal-Ace	18.0 efg	30	17.5 efgh	30
Caro-Red	15.5 efgh	25	17.0 efgh	30
Walter (a)[b]	15.4 efgh	25	15.6 efgh	25
Homestead	17.2 efgh	30	15.0 efgh	25
Campbell 1327	12.8 h	20	14.5 fgh	25
Walter (b)	14.2 fgh	25	13.7 gh	20
Average	17.6 m	30	18.7 m	30

[a] Values are averages of two lots of ten fruit each.
[b] The two sets of 'Walter' were harvested from different plantings.
[c] Percentages expressed in multiples of five for values from 10 through 50%. (USDHEW, 1973)

Table 2–β-Carotene content and percent of U.S. Recommended Daily Allowance (RDA) of vitamin A in ripe tomatoes that were harvested mature-green and ripe in 1973[a]

	Stage at harvest			
	Mature-green		Ripe	
Cultivar[b]	μg/100 gfw	% US RDA[c]	μg/100 gfw	% US RDA
Fantastic	432 bcde[d]	15	558 a	20
Double Rich	425 bcde	15	573 a	20
Rutgers	403 cde	15	490 abc	15
Homestead	396 cde	15	400 cde	15
Walter (a)[b]	394 cde	15	484 abc	15
Manapal	388 cde	15	408 cde	15
Heinz 1350	382 cde	15	524 ab	15
Cal-Ace	356 de	10	428 bcde	15
Campbell 1327	352 de	10	441 bcd	15
Walter (b)	320 e	10	446 bcd	15
Average	385 m	15	475 n	15
Caro-Red	1918	60	4190	140

[a] Values are average of two lots of ten fruit each. 'Caro-Red' was not included in statistical analysis.
[b] The two sets of 'Walter' were harvested from different plantings.
[c] Percentages expressed in multiples of five for values from 10 through 50% (USDHEW, 1973).
[d] Values not followed by common letters are significantly different (P = 0.05) (Duncan, 1955).

May lot showed differences between treated and untreated fruit, which contained 20% and 15% U.S. RDA vitamin C, respectively.

The average β-carotene contents of ripe tomatoes treated with and without supplementary ethylene were similar (Table 4). All samples had an average of 10% U.S. RDA of vitamin A.

Table 3—β-Carotene content of ripe tomatoes harvested as mature-green, breaker and ripe fruit in 1974[a]

	Stage at harvest		
	Mature-green	Breaker	Ripe
Cultivar	μg/100 gfw	μg/100 gfw	μg/100 gfw
Cal-Ace	416 def[b]	504 bcde	560 abc
MH-1	408 def	513 bcd	601 ab
Rutgers	407 ef	534 bc	651 a
Walter	370 f	440 cdef	532 bc
Average	403 m	506 n	588 o

[a] The number of lots differed with cultivar and stage of harvest. Values are averages of two to five lots of ten fruit each.
[b] Values not followed by common letters are significantly different (P = 0.05) (Duncan, 1955).

Table 4—Ascorbic acid content, percent of U.S. RDA of vitamin C, β-carotene content and percent of U.S. RDA of vitamin A in ripe 'Walter' tomatoes treated with supplementary ethylene at mature-green stage. Tomatoes from different growers at different times of the year[a]

	Ascorbic acid			
	Untreated		Treated	
Lot	mg/100 gfw	% US RDA[c]	mg/100 gfw	% US RDA
May	9.7 a[b]	15	11.8b	20
Dec.	8.6 a	15	10.0 b	15
March	11.0 a	20	12.2 a	20
	β-Carotene			
May	301 a	10	257 a	10
Dec.	287 a	10	240 a	10
March	278 a	10	275 a	10

[a] Average of fruit from four growers in May, three growers in December, and four growers in March.
[b] Values not followed by common letters within the line are significantly different (P = 0.01) (Duncan, 1955).
[c] Percentages expressed in multiples of five for values from 10 through 50% (USDHEW, 1973).

β-Carotene approaches maximum level by the time the fruit are partially ripe (Lampe and Watada, 1971), and, apparently, supplementary ethylene did not hasten color formation sufficiently for the fruit to develop ripe color before the maximum level was reached.

CONCLUSION

THE STAGE of ripeness at harvest and supplementary ethylene had only slight effects, if any, on vitamin A and C activities of ripe tomatoes. Vitamin A activity was slightly higher in ripe tomatoes that had been harvested ripe than mature-green. However, the difference between those harvested mature-green and breaker, the stages at which most fresh tomatoes are harvested, was not significant for most cultivars. Tomatoes of high vitamin activity can best be obtained by proper selection of cultivars. Dependent on the cultivar, a 100-g fruit could supply 15–50% U.S. RDA of vitamin C and 10–140% of U.S. RDA of vitamin A.

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Ms received 9/6/75; revised 11/13/75; accepted 11/20/75.

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LIPID AND FATTY ACID COMPOSITION OF CUCUMBERS AND THEIR CHANGES DURING STORAGE OF FRESH-PACK PICKLES

ABSTRACT

Changes in lipid composition and fatty acid distribution of raw cucumbers and fresh-packed pickles during storage were studied. The total lipids, neutral lipids, glycolipids and phospholipids increased when packed due to the diffusion of spice oils and loss of water. The changes in fatty acid distribution were primarily lauric, myristic, palmitic, stearic, oleic, linoleic, linolenic, tricosanoic, tricosenoic, lignoceric, and nervonic acids. Palmitic and linolenic acids were two predominant fatty acids in all fractions. The presence of short chain fatty acids in pickles due possibly to degradation could play a role in flavor changes during storage.

INTRODUCTION

THE PROMULGATION of nutritional labeling (FDA, 1973) has made ascertaining the chemical composition of each commodity a necessity, because the consumer is becoming more aware of the various components of food and their role in the diet. This also led to the formation of a Nutrient Data Bank (Rizek and Murphy, 1973). The USDA Nutrient Data Research Center is actively collecting and tabulating nutrient data (Kinsella, 1975; Kinsella et al., 1975). Quantitative data on lipids and fatty acids of fresh and processed fruits and vegetables are urgently needed since there is little literature available.

Pickles are an American favorite as evidenced by the continued increase in per capita consumption. While the amount of pickles in our daily diet is increasing, little has been published on the lipid content of pickles or changes in fatty acid composition which occur during manufacture and storage.

Vorbeck et al. (1963) reported on lipid alterations during the fermentation of cabbage. Peng (1974; 1975) also studied cabbage lipids and their changes during fermentation, processing, and storage. Kubo et al. (1974) investigated the organic acids of cucumbers after storage and Kinsella (1971) analyzed cucumber lipids.

Early work on the chemical changes and lipid alterations in fermenting cucumbers were conducted by Pederson et al. (1964). They found marked changes in all lipid fractions during fermentation from both good and bloated dill pickles. No published data are available on fresh-packed dill pickles.

The present work was undertaken to investigate the lipid composition of cucumbers and the changes which occurred in fresh-pack dill pickles during subsequent storage. The objective was to examine cucumbers and fresh-pack pickles at predetermined intervals and to analyze changes in lipid classes and fatty acid composition during storage.

EXPERIMENTAL

Materials

Cucumbers (*Cucumis sativa*, L.) and fresh-pack whole Kosher style dills were provided by The H.W. Madison Div. of The J.M. Smucker Co., Medina, Ohio. The raw stock was size graded and 3 B's (1-3/4–2 in. diam) were used for this product. Samples were allowed to age 2 wk for the equilibration of all ingredients. While most of the sample was stored at room temperature, a portion was stored in a refrigerator for comparison. Samples were analyzed at 2-, 4-, 8-, and 12-month intervals.

Lipid extraction

Duplicate samples (200g) were blended with silicic acid and Celite (Peng, 1974) and filtered to remove as much water as possible. The sample pad was extracted twice with Folch reagent (Folch et al., 1957), and the aqueous phase was washed twice with a small amount (30 ml) of chloroform. The combined extract was quantitatively transferred to a 1,000 ml pear-shaped separatory funnel with a Teflon stopcock. The extraction procedure as previously reported (Peng, 1974) was followed. Total lipids were determined gravimetrically after removing the solvent at a reduced pressure.

Lipid separation

Lipids were separated into three classes by two chromatographic columns. The nonpolar lipid fraction was isolated from polar lipids by silicic acid (Hirsch and Ahrens, 1958; Rouser et al., 1967a) in a 1.1 cm diam glass column with a 250 ml reservoir flask at a sample loading ratio of 1:50 (wt/wt) adsorbent by eluting chloroform and methanol, respectively. The flow rate throughout was 0.5 ml/min and the elution ratio of solvent/g adsorbent was 25 ml. Glycolipids were separated from phospholipids by a Florisil column (Rouser et al., 1967b; Radin, 1969) with acetone (60 ml/g adsorbent) and methanol (25 ml/g adsorbent) respectively. Solvent was removed with the aid of an evaporator at a reduced pressure. Each sample was stored in a vacuum desiccator until a constant weight was obtained.

The eluent was monitored by thin-layer chromatography (TLC). Glass plates (20 × 20 cm) were coated with Silica Gel G at 250μ thickness. Chloroform was used as the developing solvent for neutral lipids and phosphomolybdic acid used as the detecting indicator. Polar lipids were developed by Lepage solvent system (1967) consisting of chloroform:acetone:methanol:acetic acid:water (60:20:10:10:3, v/v) and identified by α-naphthol-sulfuric acid (Siakotos and Rouser, 1965).

Gas-liquid chromatography (GLC) analysis

Fatty acid composition was analyzed by a Packard model 409 Becker Gas Chromatograph (Packard Instruments, Downers Grove, Ill.) equipped with a flame ionization detector, Bristol dynamaster recorder, and disc chart integrator. Methyl ester derivatives of fatty acids were prepared by boron-trifluoride methanol (Metcalfe et al., 1966). A stainless steel column, 8 ft × 1/8 in. OD, packed with 15% by weight of diethyleneglycol succinate (DEGS) and 1% by weight of phosphoric acid on acid-washed Chromosorb W, 80/100 mesh (Applied Science Labs., State College, Pa.). Appropriate operating conditions for the methyl esters were: column temperature, 190°C; detector and injection port temperature, 210°C; carrier gas (nitrogen) flow rate, 20 ml/min; and chart speed, 1 in./min. Qualitative identification of each peak on the chromatogram was made by comparing the relative retention times of reference methyl esters and by their linear relationships when relative retention times of a homologous series of fatty acid methyl esters were plotted on a semilog paper against carbon numbers (Woodford and Van Gent, 1960). The quantitative distribution of each peak was measured by the integrator counts. The ratios of the area of each peak to the sum of the areas of all component peaks gave the percent fatty acid composition. Since GLC area percent may provide a misleading result, a weight per 100g raw sample was adopted, thus the individual peak area percentage was directly converted to the weight distribution as mg/100g sample without correction because of the linear response of the flame ionization detector to a homologous series of fatty acid methyl esters (Ackman, 1968; Dietz, 1967; Wilson et al., 1966).

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RESULTS & DISCUSSION

Changes in lipid content

Since cucumbers used for fresh-pack whole Kosher style dills have been blanched and pasteurized, any changes should not be due to microbial metabolism taking place. The change of total lipids from 138.7 mg to 204.2 mg per 100g sample (Table 1), an increase of 65.5 mg in 12 months, could be from the diffusion of oils-containing garlic and dill into the pickles and a possible loss of solids from pickle to the brine during storage. The largest increase in total lipids, 47.7 mg, occurred in the equilibration period. The most obvious explanation of this change would be due to the loss of water from the raw cucumbers (95.8%) due to blanching (92.8% in packed

Table 1—Lipid content of cucumber and pickles (mg/100g sample) (dry basis)[a]

Storage time	Total lipids		Neutral lipids		Glycolipids		Phospholipids	
	wt	%	wt	%	wt	%	wt	%
Cucumber	138.7	0.14	41.1	29.6	83.2	60.0	14.4	10.4
0-month	186.4	0.19	46.0	24.7	100.1	53.7	40.3	21.6
2-month	191.8	0.19	48.3	25.2	108.2	56.4	35.3	18.4
4-month	191.1	0.19	52.5	27.5	101.7	53.2	36.9	19.3
8-month	190.6	0.19	65.9	34.6	100.5	52.7	24.2	12.7
12-month	204.2	0.20	79.6	39.0	104.4	51.1	20.2	9.9

[a] Average of duplicate samples

Table 2—Fatty acid composition of cucumber and pickle lipids during storage (mg/100g sample)

Fatty acid[a]	Cucumber	Pickle				
		0-month	2-month	4-month	8-month	12-month
8:0	–	–	–	3.6	3.0	0.2
9:0	–	–	–	5.9	7.1	4.5
10:0	–	–	13.4	1.9	1.5	1.0
11:0	–	0.6	–	–	0.8	0.8
11:1	0.6	2.1	0.8	–	–	–
12:0	2.1	1.7	1.2	2.1	1.9	1.0
12:1	2.5	2.1	2.3	–	–	0.6
13:0	1.7	1.7	2.5	2.1	2.1	1.2
?	–	–	0.9	–	–	–
13:1	1.5	2.6	2.3	2.3	2.3	–
14:0	0.8	1.5	1.5	1.7	1.3	2.9
14:1	0.7	0.7	0.6	1.5	0.9	–
?	0.7	–	–	–	–	–
15:0	1.2	1.3	0.8	1.1	0.9	1.4
15:1	0.7	0.6	0.4	1.1	0.4	0.8
16:0	27.5	32.2	38.7	28.3	35.3	20.6
16:1	0.7	1.3	1.2	2.1	2.7	0.8
17:0	0.7	1.1	1.7	1.3	1.3	1.4
17:1	0.9	0.9	1.5	0.8	0.8	0.8
18:0	4.2	10.1	5.0	8.6	9.0	9.6
18:1	2.8	7.6	5.0	8.3	6.3	8.4
18:2	22.7	34.8	29.1	27.7	23.3	20.6
20:0	1.9	2.1	0.2	–	–	–
18:3	45.8	69.5	61.0	50.5	55.1	38.6
21:0	3.9	4.1	0.4	4.6	3.4	7.8
21:1	–	–	–	11.1	8.2	17.6
22:0	3.3	2.8	–	5.7	4.2	11.0
22:1	–	–	0.6	–	–	17.4
23:0	3.9	4.1	18.4	10.3	9.5	8.0
23:1	–	0.9	2.3	2.9	1.3	12.7
24:0	7.9	–	–	4.6	8.0	9.2
24:1	–	–	–	1.0		5.3

[a] Carbon number: number of double bond

Table 3—Fatty acid composition of neutral lipids in cucumber and pickles (mg/100g sample)

Fatty acid[a]	Cucumber	Pickle				
		0-month	2-month	4-month	8-month	12-month
9:0	–	–	0.3	1.0	1.2	0.6
10:0	0.7	7.7	2.8	3.2	4.5	3.3
11:0	–	–	0.6	0.8	1.3	0.3
11:1	–	–	1.0	–	–	–
12:0	0.7	1.2	0.9	1.0	0.9	0.6
12:1	0.7	0.7	0.8	–	–	–
13:0	0.4	0.9	0.5	1.1	1.2	0.7
?	0.4	–	–	–	–	–
13:1	0.6	0.6	1.4	0.9	1.1	–
14:0	0.5	1.5	1.0	0.8	0.9	1.5
14:1	0.5	0.5	0.8	0.7	0.6	–
?	0.5	0.3	–	–	–	–
15:0	0.5	0.7	0.8	0.5	0.5	1.0
15:1	0.6	0.5	0.8	1.0	0.9	1.0
16:0	4.7	7.0	9.1	8.7	10.9	8.9
16:1	0.3	0.2	0.6	0.7	1.1	–
17:0	0.2	0.2	0.9	0.5	0.8	0.9
16:2	–	0.1	0.2	0.5	–	–
17:1	0.2	0.3	0.8	0.8	1.1	1.4
18:0	0.8	0.9	2.5	2.5	3.6	3.6
18:1	0.5	0.6	1.6	1.7	1.6	2.3
19:0	0.2	–	–	–	–	–
18:2	1.6	0.6	5.0	4.7	3.4	3.6
20:0	–	0.3	0.1	0.5	0.6	–
18:3	12.5	16.7	9.7	11.0	13.1	11.0
21:0	0.7	1.3	0.2	1.2	1.0	–
21:1	0.4	0.4	0.1	0.6	1.3	1.4
22:0	0.5	1.7	0.3	0.8	0.8	1.6
22:1	0.6	–	–	–	0.3	2.6
23:0	–	0.8	0.3	0.3	0.5	1.2
23:1	0.5	–	0.1	0.7	0.5	1.5
?	–	–	0.2	–	0.3	–
24:0	11.3	0.3	3.7	6.3	11.9	14.4
24:1	–	–	1.2	–	–	14.0
25:0(?)	–	–	–	–	–	2.2

[a] Carbon number: number of double bond

pickles). Total lipids and moisture content remained almost constant for the next 12 months. This is a good evidence to prove that diffusion and dehydration occurred.

Total lipids found in raw cucumbers were similar to Kinsella's finding (1971), 103 mg/100g sample, but the distribution among the three lipid classes was quite different. Neutral lipids, glycolipids, and phospholipids comprised 26.6% (41.1 mg), 60.0% (83.2 mg) and 10.4% (14.4 mg) in this study as compared 39, 15 and 49% reported by Kinsella (1971). This discrepancy could be due to differences in cucumber cultivars and the cultural practices utilized in different geographical locations as well as differences due to environmental conditions.

Neutral lipids increased throughout the entire experiment. Glycolipids and phospholipids increased after the equilibration period. The increase in all classes over that found in raw cucumbers was probably moisture lost from the vegetable due to blanching and pasteurization.

The ratio of nonpolar to polar lipids was decreased after packing, then increased with storage time. The decrease in polar lipids may be attributed to the subsequent breakdown or loss into the brine, the former may account for a possible source of flavor changes.

The distribution of fatty acids from the total lipids found in this study (Table 2) is representative of fresh-packed pickle composition. Alterations may occur through the use of different spice oils. Although the types of fatty acids were similar, considerable differences were observed in their quantitative distribution at different storage times among different lipid classes. Packing seemed to be a major factor causing the alteration of lipid and fatty acid composition. Total unsaturated fatty acids appeared to change more than the saturated acids during storage. The former occupied a higher proportion of the total lipids. Neutral and phospholipids contained less unsaturated acids while less saturated acids were found in the glycolipid fraction.

The six determinations revealed that the fatty acid composition was typical of plant lipids (Hitchcock and Nichols, 1971) containing mainly lauric (12:0), myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids, and some minor acids, such as capric (10:0), palmitoleic (16:1), margaric (17:0), arachidic (20:0), behenic (22:0), erucic (22:1), tricosanoic (23:0), tricosenoic (23:1), lignoceric (24:0), and nervonic (24:1) acids. The predominant fatty acids; linoleic and linolenic increased approximately 50% after equilibration and gradually decreased with storage. Palmitic, stearic, and oleic acids contents varied. Other long chain fatty acids were inconsistant.

The gross changes of fatty acids in the neutral lipid fraction (Table 3) illustrated that the major fatty acids in fresh

Table 4–Fatty acid composition of glycolipids in cucumber and pickles (mg/100g sample)

Fatty acid[a]	Cucumber	Pickle 0-month	2-month	4-month	8-month	12-month
8:0	–	–	–	4.1	3.1	2.3
10:0	–	–	8.6	15.7	22.3	14.2
?	–	–	–	–	–	1.1
12:0	6.1	3.5	3.6	3.2	3.7	2.3
12:1	–	1.8	1.5	–	–	2.7
13:0	–	2.2	1.4	3.8	3.6	–
13:1	4.4	4.2	4.1	2.4	2.8	–
14:0	2.8	1.5	2.5	1.3	2.0	2.3
14:1	3.5	3.2	3.5	2.5	3.1	–
?	3.2	–	–	–	–	–
15:0	2.2	2.1	0.5	–	–	2.9
15:1	–	0.9	0.3	–	–	1.6
16:0	22.1	21.4	16.0	9.1	10.1	12.2
16:1	1.6	0.8	1.3	1.3	0.9	1.8
17:0	1.0	0.3	0.8	0.9	0.8	1.9
17:1	0.8	1.3	1.6	0.9	1.2	1.9
18:0	4.1	3.0	3.5	2.9	3.0	4.8
18:1	1.6	1.6	2.6	2.3	2.0	2.8
18:2	1.3	0.9	1.1	2.5	1.5	2.2
20:0	–	–	0.4	0.3	–	–
18:3	13.6	45.5	47.0	44.0	38.2	34.9
21:0	2.3	1.0	2.4	0.5	–	4.4
21:1	–	–	–	1.1	1.6	–
22:0	–	2.7	2.2	1.4	–	0.9
22:1	–	–	0.4	–	–	–
23:0	9.3	–	–	0.4	–	2.3
23:1	3.3	2.2	2.9	1.1	0.6	4.9

[a] Carbon number: number of double bond

Table 5–Fatty acid composition of phospholipids in cucumber and pickles (mg/100g sample)

Fatty acid[a]	Cucumber	Pickle 0-month	2-month	4-month	8-month	12-month
8:0	–	–	–	1.6	–	–
9:0	–	0.6	3.1	4.1	3.0	1.3
10:0	–	1.7	0.8	1.8	1.6	0.4
11:0	0.2	0.4	0.4	–	–	0.2
11:1	–	1.4	0.5	–	–	0.1
12:0	0.5	0.7	0.5	1.2	0.8	0.3
12:1	0.3	0.8	0.3	–	–	0.7
13:0	0.3	0.5	0.3	1.5	1.0	–
13:1	0.6	1.8	0.7	1.0	0.7	–
14:0	0.3	0.6	0.5	0.7	0.5	0.5
?	0.2	–	–	–	–	–
14:1	0.3	0.7	0.5	0.7	0.7	–
15:0	0.4	0.7	0.7	0.5	0.7	0.3
15:1	0.1	0.6	0.2	0.5	–	0.3
16:0	3.1	14.3	13.2	13.2	8.2	5.1
16:1	0.3	0.6	0.5	0.5	0.9	0.6
17:0	0.2	0.4	0.1	0.4	0.6	0.4
16:2	0.2	0.5	0.1	0.4	–	–
17:1	0.3	1.1	1.1	1.2	0.7	1.0
18:0	1.2	2.7	4.0	3.0	1.2	1.7
18:1	0.8	1.2	0.7	0.5	0.5	0.4
19:0	–	–	–	–	–	0.2
18:2	0.4	1.8	1.2	0.3	–	0.2
20:0	0.6	0.7	0.5	0.2	–	–
18:3	2.9	1.4	2.5	2.4	1.7	2.2
21:0	0.6	1.3	0.8	0.5	–	1.0
21:1	–	–	–	0.3	–	–
22:0	0.4	1.3	1.0	0.3	1.4	0.4
23:0	–	0.6	1.1	0.1	–	0.8
23:1	0.2	1.9	–	–	–	–
24:0	–	–	–	–	–	1.3
24:1	–	–	–	–	–	0.8

[a] Carbon number: number of double bond

cucumbers were palmitic, linoleic, linolenic, and lignoceric acids. After packing, most of these same fatty acids increased except linoleic and lignoceric acids. After 12-month storage all major fatty acids generally increased in quantity. Higher amounts of lignoceric and nervonic acids were noted.

Higher glycolipid content is typical of a photosynthesizing green plant. The fatty acid distribution of the glycolipid fraction (Table 4) was predominated by palmitic, linolenic, and tricosanoic acids, followed by lauric, tridecenoic (13:1), stearic, and tricosenoic acids. Packing and storage caused a considerable decrease of most fatty acids. Only linolenic acid was increased and by more than three-times after processing. However, it decreased throughout storage but was the predominant fatty acid throughout storage.

The phospholipid fraction was the least of the lipid classes in cucumber and pickles. The concentration of all fatty acids increased after packing (Table 5) except linolenic acid, which agreed with the general pattern reported for cabbage and sauerkraut (Peng, 1974). During storage, palmitic, oleic, and linoleic acids decreased consistantly, while lauric, myristic, stearic, and linolenic acids varied.

Throughout the entire period of storage, palmitic and linolenic were the two predominant fatty acids distributed among all four lipid fractions and found in higher concentrations with a random alteration.

It is also interesting to note that the shorter chain fatty acids (8:0 to 10:0) did not appear in the raw cucumber except a small amount of capric (10:0) acid in the neutral lipid fraction (Table 3). The presence of these shorter chain fatty acids in pickles would indicate degradation of long chain acids and could influence the flavor of the product during storage.

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Ms received 12/13/75; revised 2/14/76; accepted 2/16/76.

Approved for publication as Journal Article No. 134-75 of the Ohio Agricultural Research and Development Center, Wooster, OH 44691.

The authors thank The H.W. Madison Div. of The J.M. Smucker Co., Medina, Ohio for providing and processing cucumber and pickle samples for this study, and Miss M.L. Yao for computing GLC data.

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EFFECT OF COLLAGEN LEVELS AND SARCOMERE SHORTENING ON MUSCLE TENDERNESS

ABSTRACT

A study was conducted using two muscles of different connective tissue content [13.13 ± 3.11 and 2.47 ± 0.95 mg collagen/g of muscle for sternomandibularis (S) and psoas major (PM) muscles, respectively], which had shortened to various sarcomere lengths (1.35–2.6 μm and 1.7–3.25 μm for the S and PM muscles, respectively). Differences in structural preservation of the mitochondria, triads and Z-lines were noted for the stretched and contracted samples of both muscles. The difference in connective tissue of the two muscles was related to the differences in tenderness of these muscles at all sarcomere lengths. However, the difference in tenderness of these muscles was not constant at all sarcomere lengths, with the S muscle decreasing in shear force at a faster rate due to increasing sarcomere length. This phenomena is probably due to a toughening of both connective tissue and muscle fibers as the S muscle shortens, whereas, in the PM muscle, only the muscle fibers cause a toughening due to shortening. This study also indicates the effectiveness of using a trained sensory panel to detect connective tissue and muscle fiber tenderness separately.

INTRODUCTION

TWO OF THE MAJOR contributors to muscle tenderness are: the state and content of connective tissue, and the structure and state of contraction of the myofibrils (Locker, 1960; Cover et al., 1962; Machlic and Draudt, 1963; Bouton et al., 1973a; Cross et al., 1973; McCrae and Paul, 1974). Considerable research has been conducted on the relationship of the shortening of muscle fibers to meat tenderness (Locker, 1960; Herring et al., 1965; Hostetler et al., 1972, 1975; Marsh and Carse, 1974), which has conclusively demonstrated that as muscle fibers shorten, tenderness of muscle decreases. In comparison, there is very little research that has been accomplished concerning the relationship of muscle shortening to the connective tissue component of meat tenderness. The research of Kruggel and Field (1971) and Pfeiffer et al. (1972) shows that the amount of extractable low molecular weight collagen subunits is increased by stretching a muscle. Research by O'Shea et al. (1974) shows a change in thermal stability of collagen from stretched and shortened muscle but that these changes were not related to shear force. However, the research of Bouton et al. (1973b) indicates that there is a difference in adhesion values of a muscle at widely different sarcomere lengths and that these differences could be related to connective tissue strength.

Thus, the objectives of this study were to determine the effect of muscle shortening on tenderness of muscles of different connective tissue content.

EXPERIMENTAL

32 STERNOMANDIBULARIS (S) and 29 psoas major (PM) muscles were used in this study and represent muscles with high and low amounts of connective tissue, respectively. The length (100% resting length) of the sternomandibularis muscles was measured immediately after bleeding the animal, with the muscle attached at its origin and insertion. The head of the animal was in a position that caused the muscle to be stretched. The length (100% resting length) of a section of the psoas major muscle was measured while the carcass was suspended from the achilles tendon which also caused this muscle to be stretched. The S and PM muscles were excised at approximately 5 and 25 min postmortem, respectively, and tied at a length which was from 50–100% of the resting length. Muscles were then covered with PVC film, placed in a cooler at 2°C and allowed to shorten to the length at which they were tied to produce a variation in sarcomere length.

After 48 hr in the cold room, samples were removed from each muscle for sarcomere length determination on raw muscle samples by measuring the length of 10 sarcomeres in each of 25 myofibrils from each muscle (Hostetler et al., 1975). Samples were also embedded for transmission electron microscopy by the methods described by Dutson (1974). The embedded samples were sectioned at 60 nm for electron microscopy using an LKB ultramicrotome. Electron micrographs were taken using a Hitachi HU-11-E electron microscope. Tenderness of muscle fibers (ease of fragmentation) and connective tissue (connective tissue softness) was measured by a sensory panel. Tenderness was also measured by Warner-Bratzler Shear (Hostetler et al., 1975). The amount of collagen in these two muscles was measured by hydroxyproline analysis according to the procedures of Woessner (1961).

Analysis of variance, linear regression and correlations were determined according to Dixon and Massey (1957).

RESULTS & DISCUSSION

THERE WAS A significant difference ($P < 0.001$) in the amount of collagen for the two muscles studied, with the PM muscle containing 2.47 ± 0.95 mg of collagen/g of muscle tissue and the S muscle containing 13.13 ± 3.11 mg/g.

Restraining muscles at various percentages of rest length was an effective means of producing a variation in shortening as evidenced by a range in sarcomere length of 1.7–3.25 μm for the PM muscles and 1.35–2.6 μm for the S muscles (Fig. 5–7).

Electron micrographs of representative PM and S muscles at

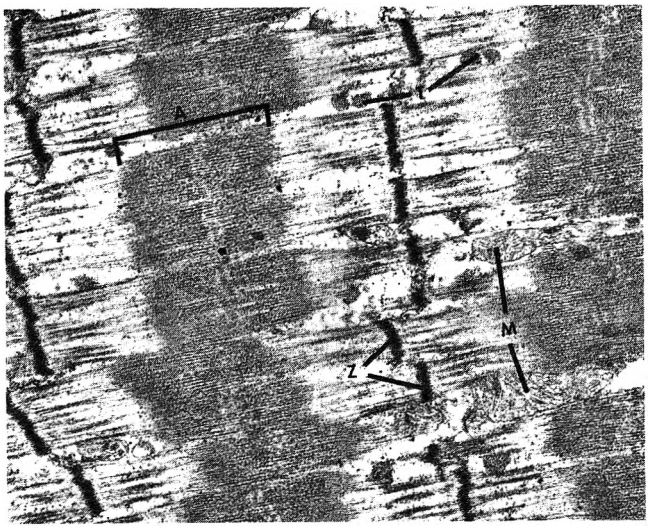

Fig. 1—Electron micrograph taken from a stretched psoas major muscle. A = A band, Z = Z line, M = mitochondria and T = triads. X 16,125.

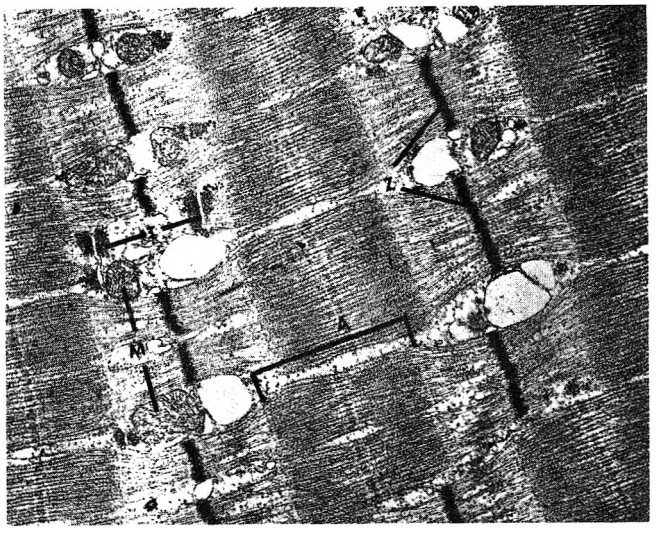

Fig. 3—Electron micrograph taken from a stretched sternomandibularis muscle. A = A band, Z = Z line, M = mitochondria and T = triads. X 16,125.

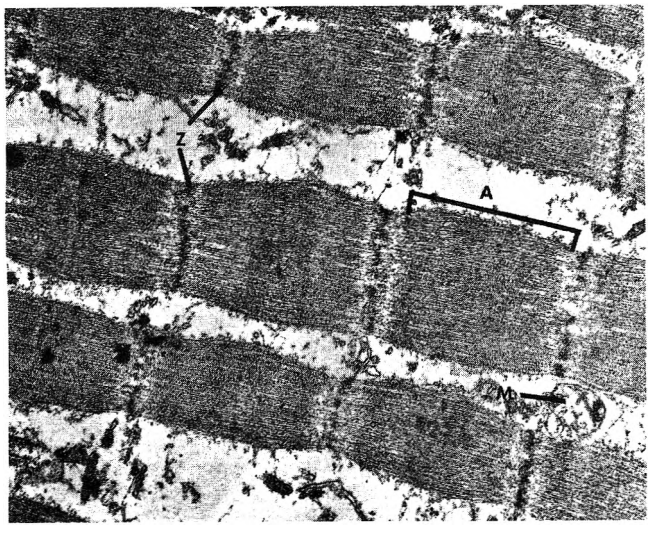

Fig. 2—Electron micrograph taken from a contracted psoas major muscle. A = A band, Z = Z line and M = mitochondria. X 16,125.

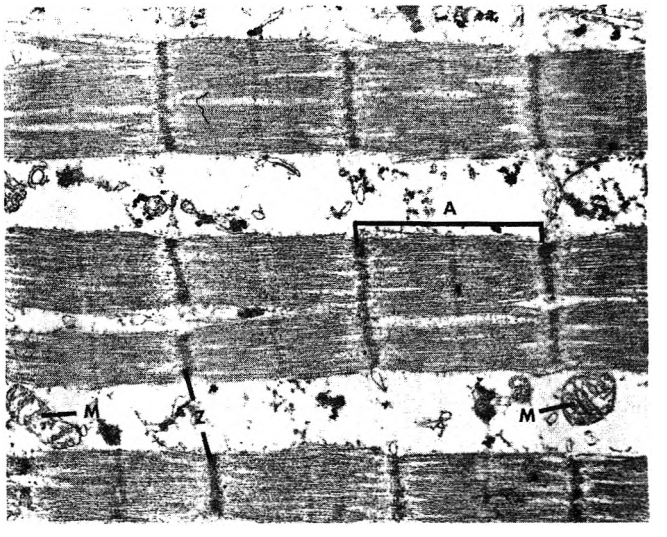

Fig. 4—Electron micrograph taken from a contracted sternomandibularis muscle. A = A band, Z = Z line and M = mitochondria. X 16,125.

50 and 100% of resting length are presented in Figures 1–4. As can be seen from these figures, the PM muscle was in a more stretched position at 100% of resting length than was the S muscle. This is also evidenced by the longer sarcomere lengths found in the PM muscles (Fig. 5–7). In comparing the data for the S muscles with that obtained by Marsh and Carse (1974) for the same muscle, it is evident that the resting length muscles in the present study (sarcomere length of 2.6 μm) are more elongated than the resting length muscles of Marsh and Carse (1974) (sarcomere length calculated to be 2.1 μm). It is interesting to note that there was no evidence of nonlinearity for shear force vs sarcomere length in this study whereas nonlinearity was evident in the study of Marsh and Carse (1974). This could possibly be due to the differences in amount of stretch.

Both the PM and S muscles held in a stretched position (Fig. 1 and 3) show greater structural preservation of the triads, mitochondria and particularly the Z-line when compared to the shortened muscles (Fig. 2 and 4).

The difference in Z-line integrity between stretched and contracted samples is interesting since numerous researchers (Goll et al., 1970; Henderson et al., 1970; Dutson et al., 1974) have shown that one of the major postmortem changes that occur in myofibrils is disruption of the Z-line. Further research in this area is needed to determine if contraction of postmortem muscle might actually be a factor in the initiation of Z-line disruption. According to the work of Marsh et al. (1974), there is a possibility that the myosin filaments are a factor in disruption of Z-lines of extremely shortened muscle. There is also greater separation between the myofibrils of shortened muscle, which is probably due to an increase in diameter of the muscle fibers (fiber volume must remain unchanged), while the diameter of the myofibrils doesn't change appreciably.

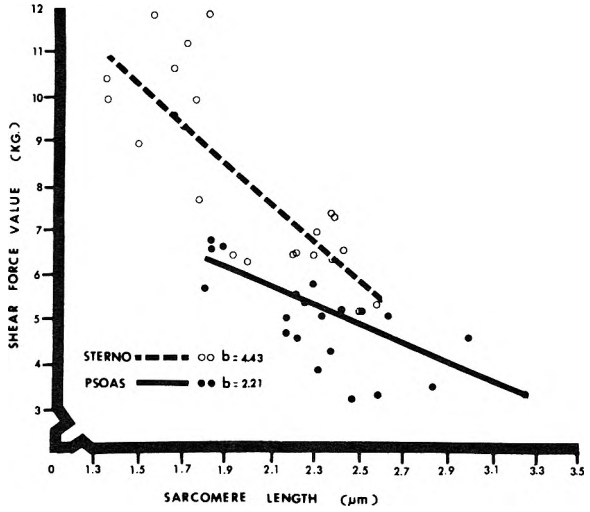

Fig. 5–Regression lines for shear force vs sarcomere length of the psoas major and sternomandibularis muscles.

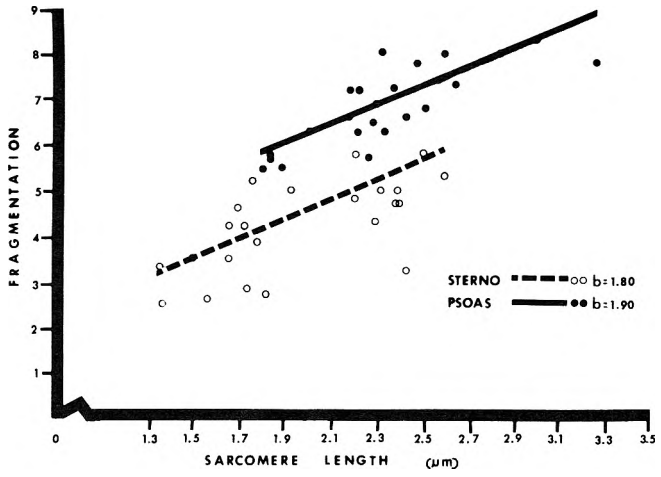

Fig. 6–Regression lines for muscle fiber fragmentation vs sarcomere length of the psoas major and sternomandibularis muscles.

Table 1–Correlation coefficients for sarcomere length vs shear force, ease of fragmentation and connective tissue softness

	Sarcomere length vs		
Muscle	Shear force	Fragmentation	Connective tissue softness
PM	−0.72***	0.77***	0.19
S	−0.80***	0.64***	0.49*

* Significant at the $P < 0.05$ level
*** Significant at the $P < 0.001$ level

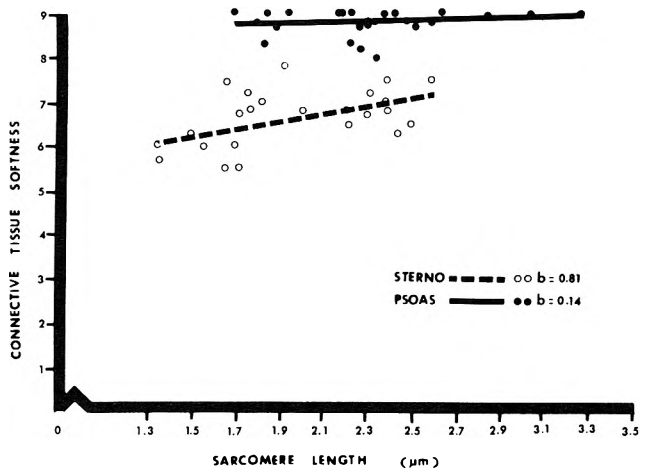

Fig. 7–Regression lines for connective tissue softness vs sarcomere length of the psoas major and sternomandibularis muscles.

Graphs of shear force, muscle fiber fragmentation and connective tissue softness vs sarcomere length are shown in Figures 5, 6 and 7, respectively. For the data presented in these graphs, there was no evidence of nonlinearity by the methods of Dixon and Massey (1957), so, linear regression lines are presented. Regression lines of shear force vs sarcomere length for the PM and S muscles (Fig. 5) indicate that the S muscles had a shear force greater than the PM muscles at all sarcomere lengths. This is probably a result of the greater amount of connective tissue present in the S muscles. However, the slope of the regression line for the S muscles is greater than that for the PM muscles (slope for S = −9.89 and for PM = −6.44). Thus, it appears that a greater reduction in shear force is caused by stretching the S muscles than by stretching the PM muscles.

The greater rate of toughening (shear force) of the S muscles upon shortening could be due to an increase in shear resistance of both the connective tissue and the muscle fibers as the muscle shortens in the S muscle, whereas in the PM muscle, there is very little connective tissue leaving the muscle fibers as the primary factor responsible for toughening. A toughening of the connective tissue in the S muscle could be a result of an increase in its density (O'Shea et al., 1974) or to a change in its molecular properties as reported by Kruggel and Field (1971) and Pfeiffer et al. (1972). One other possibility for the differences found between the S and PM muscles is that there could be difference in the toughening characteristics of the muscle fibers of these two muscles.

A plot of muscle fiber fragmentation, as measured by a sensory panel, vs sarcomere length for the S and PM muscles is presented in Figure 6. The slope of the regression line for the PM muscle is 1.8 and the slope of the regression line for the S muscle 1.9, indicating that the muscle fibers of these two muscles toughen at the same rate due to shortening.

A plot of connective tissue softness, as measured by a sensory panel, vs sarcomere length is presented in Figure 7 for the S and PM muscles. There is very little change in toughness of connective tissue of the PM muscles due to shortening while shortening of the S muscles caused an increase in the toughness of the connective tissue as perceived by the sensory panel. By comparing Figures 5, 6 and 7, it appears that shear force is a measurement of both connective tissue and muscle fiber toughness, whereas the sensory panel is able to separate differences in toughness of muscle fibers and connective tissue.

Correlation coefficients between sarcomere length and shear force, ease of fragmentation, and connective tissue soft-

ness, are presented in Table 1 for the PM and S muscles. There was a significant decrease in shear force and an increase in fragmentation index with increases in sarcomere length for the PM muscle, while there was no significant change in connective tissue softness in this muscle. There was also a significant decrease in shear force and an increase in ease of fragmentation for the S muscle with increasing sarcomere length. In addition, there was a significant increase in connective tissue softness with increasing sarcomere length for the S muscle, indicating that the tenderness of the connective tissue in this muscle is increased by preventing shortening. It is also interesting to note that the magnitude of the correlation between sarcomere length and shear force is greater for the S muscle than the PM muscle, whereas, for fragmentation, the magnitude of the correlation is less for the S muscle. Also, the correlation between sarcomere length and connective tissue softness for the S muscle is considerably greater than that for the PM muscle. This again indicates that a trained sensory panel is able to distinguish between the tenderness of muscle fibers and the tenderness of connective tissue, whereas shear force is a measure of the resistance of both of these parameters.

Thus, the results of this study indicate that, for the two muscles studied, connective tissue content plays a role in the overall tenderness of a muscle at all sarcomere lengths and it is also implicated in the amount of toughening a muscle undergoes when it shortens. However, more research needs to be conducted using other muscles of differing connective tissue content to determine if this phenomenon is true for all muscles.

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Ms received 6/16/75; revised 12/19/75; accepted 12/25/75.

Presented at the 35th Annual Meeting of the Institute of Food Technologists, Chicago, Ill., June 8—12, 1975.

T.A. 11966, Texas Agricultural Experiment Station. This study was partially supported by a grant from King Ranch, Inc., Kingsville, Texas. Appreciation is expressed to B.A. Johnson for technical assistance.

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MUSCLE SAMPLES FOR SCANNING ELECTRON MICROSCOPY: PREPARATIVE TECHNIQUES AND GENERAL MORPHOLOGY

ABSTRACT

Bovine semitendinosus and longissimus dorsi, both freshly slaughtered and aged, were prepared by various techniques prior to examination by SEM. The important problems of sample preparation were freeze damage, production of surface artifacts, and tissue shrinkage. Ethanol freeze fracture of fixed material followed by critical point drying or air drying from solvent circumvented most of the difficulties and provided the most useful specimens. Cleavage of muscle tissue by freeze fracture exposed muscle cell structures for observation. Useful magnifications up to 20,000 × were obtained and identification was made of A, I and H bands, nuclei, and structures suggestive of mitochondria and muscle cell triads. The structural consequences of cold shortening and rigor were observed.

INTRODUCTION

THE STRUCTURE of muscle tissue and the changes associated with rigor and postmortem aging in various muscles have been extensively studied using conventional transmission electron microscopy (TEM) (Ramsbottom and Strandine, 1949; Stromer et al., 1967; Weidemann et al., 1967; Henderson et al., 1970; McCallister and Hadek, 1970; Dutson et al., 1974). Scanning electron microscopy (SEM) has been used to describe structures in mammalian skeletal muscle (Schaller and Powrie, 1971, 1972; Stanley and Geissinger, 1972; Eino and Stanley, 1973) and cardiac muscle (Sybers and Sheldon, 1975).

However, the SEM of muscle tissue is relatively new; there is by no means agreement among researchers concerning interpretation of several structures which appear in SEM micrographs of muscle (q.v., Sybers and Sheldon, 1975) or concerning preparative techniques for muscle samples.

Preparation of muscle tissue for viewing by SEM presents severe problems. Muscle cells seem especially sensitive to water removal and tissue shrinkage is difficult to avoid. Sample preparations involving cutting of any kind produce surface artifacts which make interpretation of micrographs difficult. Indeed, because SEM images surfaces, any abrasions of the fragile muscle specimen during conventional sample preparation limits the information from that sample. In some published micrographs, the morphology shown has clearly been affected by sample preparation.

In the course of our investigation of the relationship of muscle morphology to meat tenderness, we found it absolutely essential to find a method of sample preparation which gave reasonable, consistent preservation and gave samples for examination by SEM which were comparable in prior treatment in order to permit comparisons of structure. In the present paper, we report on the general morphology of bovine skeletal muscle as viewed by SEM after various preparative techniques. Hopefully, information presented here will provide a guide to sample preparations that produce SEM micrographs of consistently high quality which will aid in identifying and elucidating structures whose interpretation is controversial.

MATERIALS & METHODS

Muscle samples

Muscle tissue was either bovine longissimus dorsi or semitendinosus. Longissimus was obtained from a steer graded "good," 45 min postmortem (and fixed in the cold immediately), or from the same muscle stored 24 hr at 6°C. Semitendinosus was obtained from a supermarket [commercial beef is normally aged 2–4 wk at temperatures between 32° and 40°F (Ramsbotton and Strandine, 1949)]. The specimens for SEM were segments of muscle fiber bundles (1 cm^2 × 4 cm), excised intact, and small blocks (1 cm^3) of muscle, blade cut or freeze fractured to expose interior surfaces.

Fixation

Fixatives used were 2.5% glutaraldehyde in 0.1M phosphate buffer or a modified Karnovsky's-type fixative (Heald, 1971) containing 2% paraformaldehyde, 2% glutaraldehyde, and a trace of Ca^{+2} (2.5×10^{-3}% $CaCl_2$) in 0.05M phosphate. The post-fix, when used, was 1% OsO_4 in 0.05M phosphate. Fixative was used at pH 7 for freshly slaughtered muscle and pH 6 for aged muscle.

Muscle bundles were fixed overnight. Other samples, less than 1 cm on a side, were immersed in fixative for 1 hr to firm the tissue, then trimmed to a smaller size and returned to fresh fixative for another hour. These steps were repeated until the tissue pieces were the desired sizes. Continuous attention was given to the eventual surface to be viewed in the microscope. Trimming was confined to wet samples as much as possible because of extreme fragility of specimens after drying. Total fixation time for tissue blocks was about 6 hr at room temperature. All fixed samples, bundles and pieces, were rinsed in three successive changes of 0.05M phosphate buffer for 10 min each and a final water rinse. Some samples were post-fixed for 1 hr at room temperature. Water-ethanol exchange was carried out by immersing the tissue in aqueous solutions of 70, 95, 95, 100, 100% ethanol, successively, for 10 min each. Acetone, as well as other concentrations of ethanol, were used in the dehydration series for comparison. The number of steps in the series and the immersion time at each step were also varied.

Freeze fracturing

This general procedure for exposing internal surfaces of tissues was done both by fracturing from water and from ethanol. In preliminary experiments, fixed and unfixed tissue specimens (1 cm × 6 mm × 5 mm) were frozen in Freon 22 cooled to near liquid nitrogen temperature. Many samples fractured spontaneously; others were snapped with a scalpel while the sample was submerged in liquid nitrogen. If specimens were unfixed at this stage, they were dropped immediately into fixative. Some of the samples were post-fixed. The preferred method of freeze-fracture was that suggested by Humphreys et al. (1974). Ethanol-impregnated fixed tissue (1.5 × 1.5 × 3 mm) was sealed in a liquid ethanol environment within a Parafilm cylinder which had been formed around a wooden applicator stick. The packets were frozen in liquid nitrogen and, while supported on a liquid nitrogen-cooled brass block, were fractured by pressure from a sharp, cold scalpel blade. Frozen halves were returned to fluid ethanol to thaw before drying by the critical point method or at room temperature in a stream of nitrogen gas.

Drying procedures

Methods of drying tissues were freeze drying, critical point drying, and air drying at room temperature in a stream of nitrogen gas. Freeze drying was carried out by conventional lyophilization (freezing in Freon 22 at liquid nitrogen temperature and allowing to warm up fairly

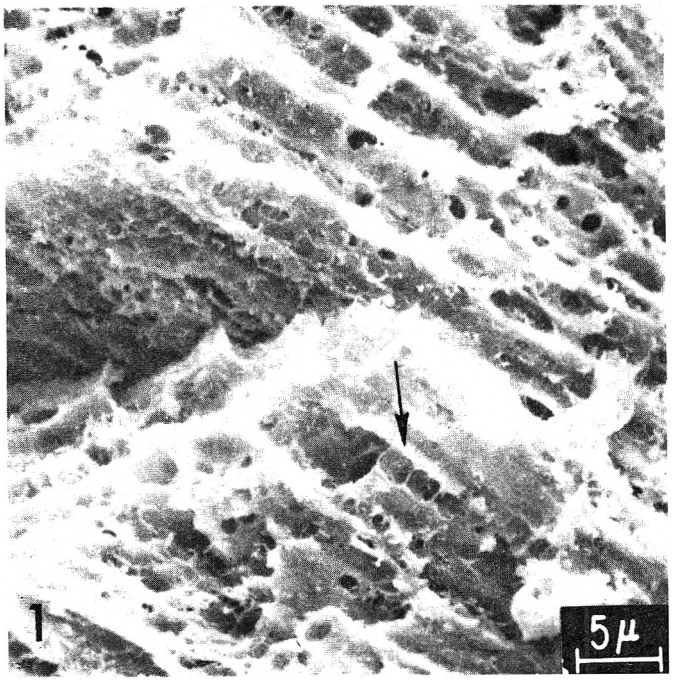

Fig. 1–Fracture surface through unfixed semitendinosus muscle fibers showing severe ice damage. Some remains of myofibrillar structure (arrow) and many ice crystal voids are seen.

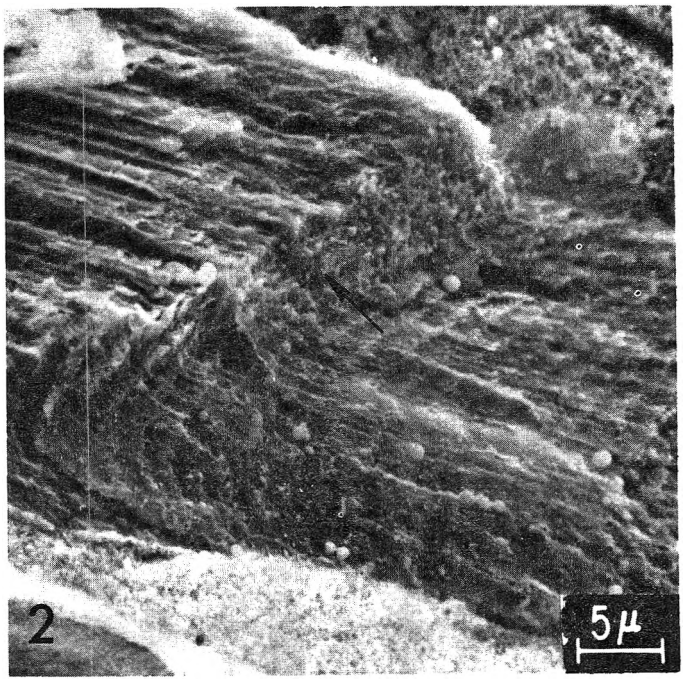

Fig. 2–Fracture surface through fixed semitendinosus muscle fibers. Myofibril structure maintained. Cleavage occurred parallel to fiber, with occasional vertical steps (arrow).

rapidly at a pressure of about 10^{-4} torr near a cold trap containing acetone-dry ice mixture; time, 2 hr) and by the conventional tissue-drying technique (freezing as above, laying samples in holes in a brass block chilled in liquid nitrogen, and placing block in a vacuum evaporator at 10^{-5} torr; time, 18 hr). The second method keeps the sample at a lower temperature (−60° to −70°C) while it dries. There is less risk of tissue damage by ice crystals reforming in the temperature range −40°C to 0°C (Boyde and Echlin, 1973).

For critical point drying, liquid CO_2 was used as transition fluid in a Denton Critical Point Drying apparatus. Samples were treated by dehydration in ethanol or acetone, followed by amyl acetate (Nemanic, 1972) or simply by dehydration followed by CO_2 exchange in the bomb (Humphreys et al., 1974). Exchange time in the bomb was determined by the size of the sample: 20 min for small samples (1 mm^3) or 45 min for larger pieces (5 mm^3). Air drying was used only with small (1 mm^3) samples which were impregnated with alcohol. Samples were placed in a shallow container which was flushed with a stream of nitrogen gas for about 2 hr.

All samples were stored in a desiccator when dry.

Mounting and metal coating for SEM

Dried tissue was attached to specimen stubs with conductive silver paint, with the surface of interest carefully oriented uppermost. Fractured surfaces when dry were usually easy to identify because they were flat and shiny when viewed through a dissecting microscope. Samples were painted on all sides except the top with conductive silver paint to minimize charging in the microscope, and were coated with approximately 150 nm of gold-palladium (60/40) on a rotary tilting stage in a vacuum evaporator. Coated samples were stored in a desiccator. Specimens were observed in a JSM-50A with an accelerating voltage of 15 kV and a specimen current of 2×10^{-12} amps.

RESULTS & DISCUSSION

Effects of preparative treatment on structure preservation

Certain biological tissues (for example, some plant tissues) can be prepared for observation by SEM without fixation. However, the results obtained with muscle tissue are critically dependent not only on the fixation but on all parts of the preparative procedure as well. The accompanying micrographs will illustrate the effects of a variety of different sample preparations on the preservation of structure as viewed by SEM.

Figure 1 shows the results of freeze-fracturing before fixing the tissue. This sample of semitendinosus was freeze fractured in water and then fixed in modified Karnovsky's fixative and freeze dried from water in the vacuum evaporator. Some ragged remains of myofibril cross structure (arrow) are the only suggestion of the well-known fiber and myofibril arrangement of muscle. Large ice crystal voids are seen throughout the tissue. Figure 2 is similar tissue fixed before fracturing and freeze drying. The fracture occurred in the direction of the fiber, but caused some cleavage in other planes as well (arrow). Myofibrillar organization has been retained with the typical transverse banding of the fiber in evidence. At greater magnification, however, the fine structure of fixed, freeze-dried tissue (not fractured) discloses the poor preservation in the myofibril structure (Mf) (Fig. 3). In some places, voids occur between myofibrils near Z-lines where the T-tubule system probably interrupted the muscle fiber. A robust-looking fiber, disrupted from its origin outside this particular muscle cell, can be seen lying across the myofibrils. It is possibly connective tissue.

Figure 4 is another sample of semitendinosus, fixed before fracturing, then freeze dried by lyophilization. This tissue was prepared essentially in the same way as the previous specimen, but is noticeably better preserved. Well-defined myofibril structure is recognizable. The sarcomeres in this micrograph measure approximately 1.6μ and zones on sarcomeres are identifiable from their topography. Sarcomeres are delimited by prominent bands (unmarked arrow) which either overlie or are a part of the Z-discs. The bands are bumpy and tubular in appearance and are continuous across the fiber. Immediately next to each Z-area are smaller raised bands in the I-band regions (arrowhead). These correspond in location to the triads (T-tubules plus sarcoplasmic reticulum cisternae) of the mam-

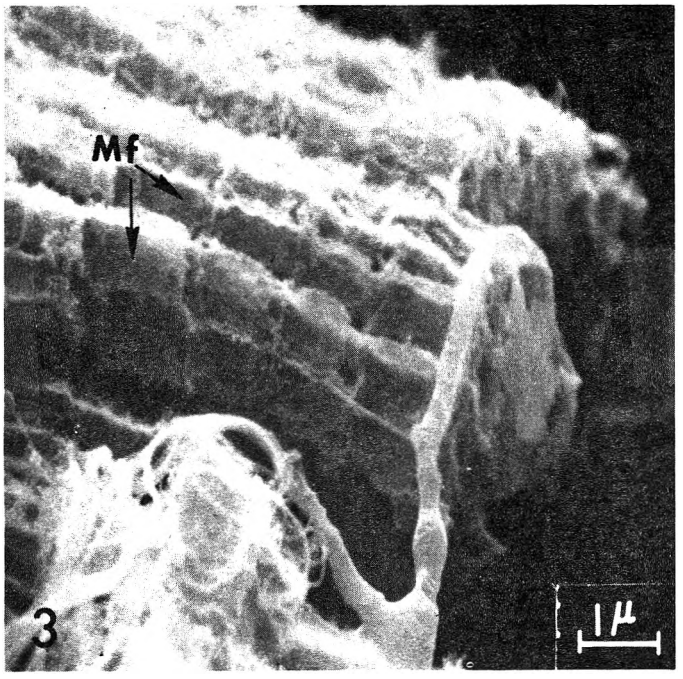

Fig. 3–Semitendinosus fixed, freeze dried. Structure recognizable but damage to myofibrils (Mf) apparent. Voids occur where T-tubules pulled away.

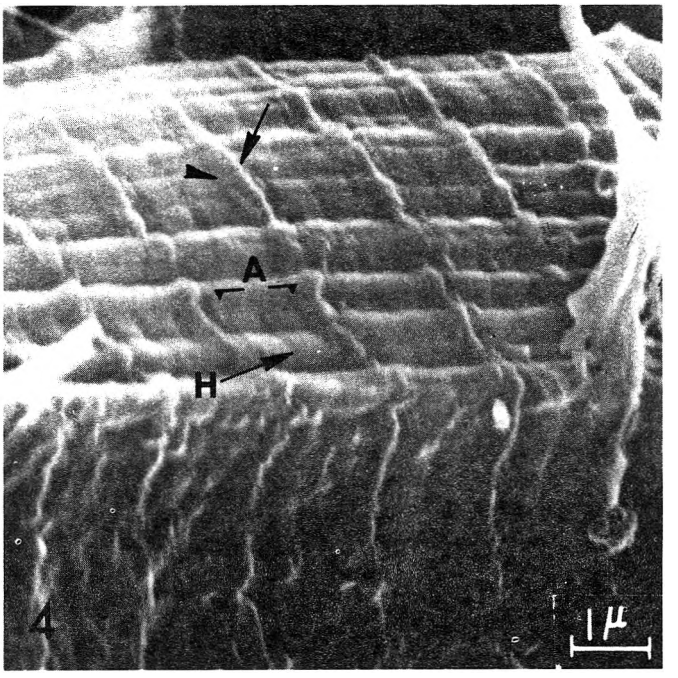

Fig. 4–Semitendinosus, fixed, freeze dried. Tissue well preserved, showing raised structures at the Z-line (unmarked arrow), A bands (A), H bands (H), and possible terminal cisternae (arrowhead).

malian muscle cell (Peachey, 1970) identifiable by transmission electron microscopy (TEM). The myosin bands (A-bands) are demarcated at their midpoints by a slight channel with a ridge running down the center (H). This depression corresponds to the H-band as defined by TEM. It is probable that this much structure is visible only because of a certain amount of shrinkage in the tissue, leaving some areas high, others low.

Post-fixation with osmium imparted a quality to samples which enhanced image contrast in the SEM. The specimen in Figure 5 was prepared by fixation, post-fixation, and freeze drying. The micrograph shows the surface of adjacent fibers of a semitendinosus bundle. The fiber surfaces are covered by endomysial collagen and, beneath that, the surface of the sarcolemma, all of which obscure underlying cell structure. It would be necessary to clear away the covering material completely, or to fracture, if underlying tissues were to be imaged. Scattered over the surface are patches of material (arrow), possibly lipid, deposited during the preparative procedure. Post-fixation was judged to be unnecessary for general sample preparation for SEM, but was useful in producing good quality micrographs.

In general, results from freeze-drying methods were quite variable. Fixation was a primary requirement, but it alone was not sufficient to ensure adequate specimen preservation when using freeze drying. One variable which was judged controllable, in this and all drying methods, was sample size. Smaller specimens were more likely to be well preserved.

Results obtained with the critical point drying technique are illustrated in the next two micrographs (Fig. 6 and 7). Figure 6 shows semitendinosus, fixed, fractured in water, and dried by the critical point method after ethanol and amyl acetate dehydration. The fracture followed fiber surfaces and occasionally stripped some contractile elements near the surface, as shown in the micrograph. The general appearance is that of a rather granular and abraded surface (unmarked arrow). Well-preserved myofibrils without much surface definition are imaged; possible mitochondria (M) can be seen in spaces between adjacent myofibrils. Figure 7 shows similar tissue prepared by ethanol fracturing and critical point drying directly from ethanol. Sarcomeres measure approximately 1.6μ. The general surface appearance is somewhat grainy or powdery with moderate surface definition. Raised structures again occur at the Z-lines and are referred to in this figure as T. I-bands (I) are faintly demarcated and the H-band areas (H) of the myosin are raised over a considerable width. Myofibrils are slightly separated from each other, except in the H-bands. In fact, adjacent myofibrils in most samples were in intimate contact at the H-band and at the Z-line. The sarcomeres are observed to narrow slightly as they approach the Z-lines, also noted by TEM by Stromer and Goll, 1967.

Comparing this micrograph to Figure 4 will point up some of the differences between well-preserved freeze dried (or air dried from solvent) and well preserved critical point dried meat tissue. Material which was freeze dried or air dried from solvent usually had better surface definition, gave "crisper," clearer micrographs, but had somewhat more shrinkage. Tissue which was critical point dried had less well defined topography, a mealy or abraded surface, but less overall shrinkage. Selection of technique depended upon the object of the particular experiment.

SEM of fresh muscle and the effects of cold shortening

The most useful preparative technique was to freeze fracture very small specimens in ethanol and either air dry or critical point dry from ethanol. This technique was employed for samples of longissimus dorsi excised at slaughter. Tissue fixed 45 min after slaughter was kept in fixative unrestrained for 24 hr at 6°C. The material was then freeze fractured in

ethanol and pieces about 1 mm^3 were air dried in a stream of nitrogen gas. The results are shown in Figure 8.

The fibers (F) in this figure lie nearly horizontal to the plane of the micrograph. The fracture has exposed several parallel fibers, 20–40μ in diameter, which have sharply defined contraction waves. The contractions occur in tandem, so that the deep clefts (unmarked arrow) appear to form nearly straight lines across the fibers. The fracture follows fiber sur-

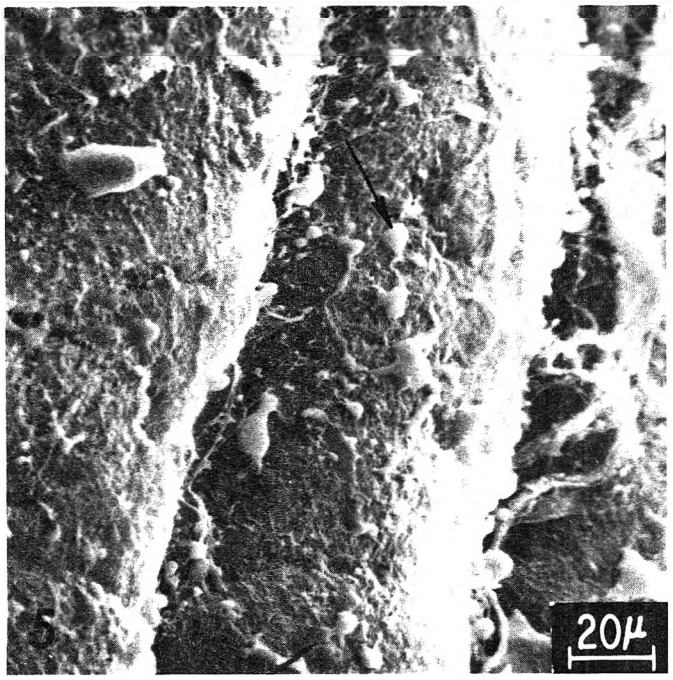

Fig. 5—Fiber surfaces of semitendinosus bundle. Collagen and other reticular material obscure underlying structure. Droplets of extraneous material (arrow), possibly lipids, seen on surface.

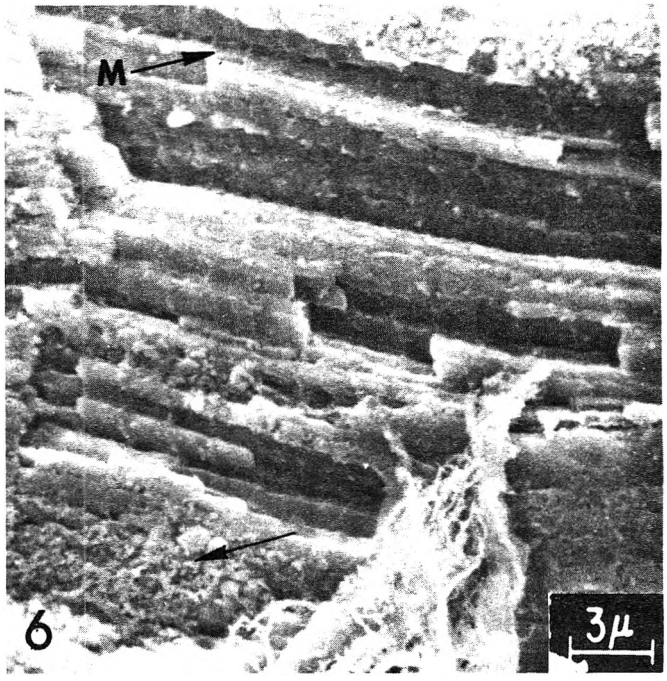

Fig. 6—Semitendinosus, fixed, fractured, and critical point dried. Fibrils well preserved without much surface definition. Mitochondri (M) visible. Some graininess in sample surface (arrow).

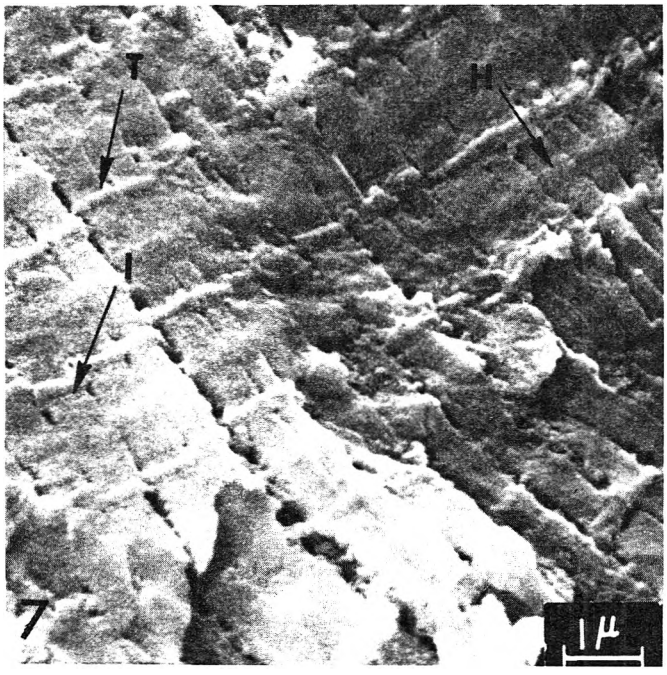

Fig. 7—Semitendinosus, critical point dried. Sarcomeres 1.6μ, with raised structures at the Z-lines (T), I-band (I) and H-band (H) raised to form ridge.

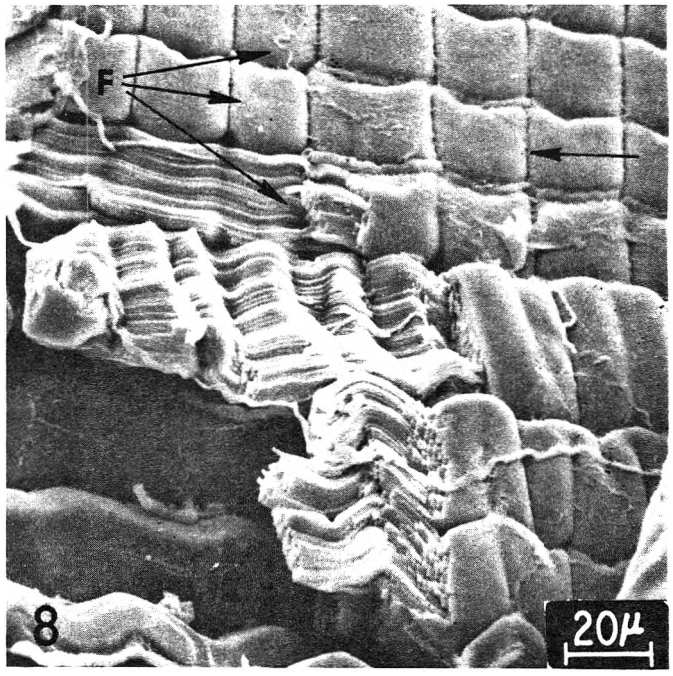

Fig. 8—Fracture through longissimus dorsi, fixed immediately after slaughter. Fiber (F) surfaces exposed, extreme contraction produced deep clefts (unmarked arrow), with some breakage of myofibrils.

faces except in the lower portion of the micrograph where fibers have been sheared longitudinally. Orderly rows of myofibrils in that area repeat the wave pattern of the surface. Some rupture of myofibrils is observed in the cleft area of the wave pattern. The periodicity of the wave pattern is 20–30μ in this case.

The presence of well defined contraction waves in muscle tissue is associated with cold-shortening. This condition occurs

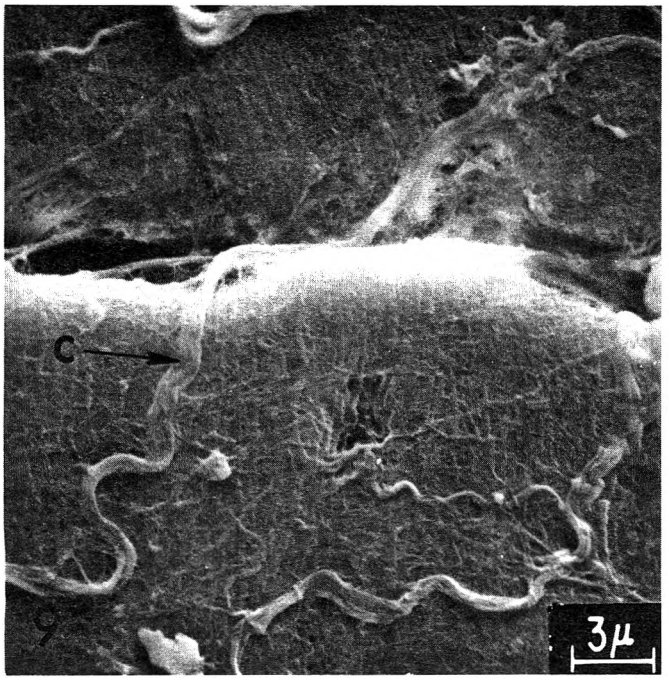

Fig. 9—Exposed surface of cold-shortened longissimus dorsi fibers, fixed immediately after slaughter. Collagen fibers (C) and reticular fibers cover contractile elements.

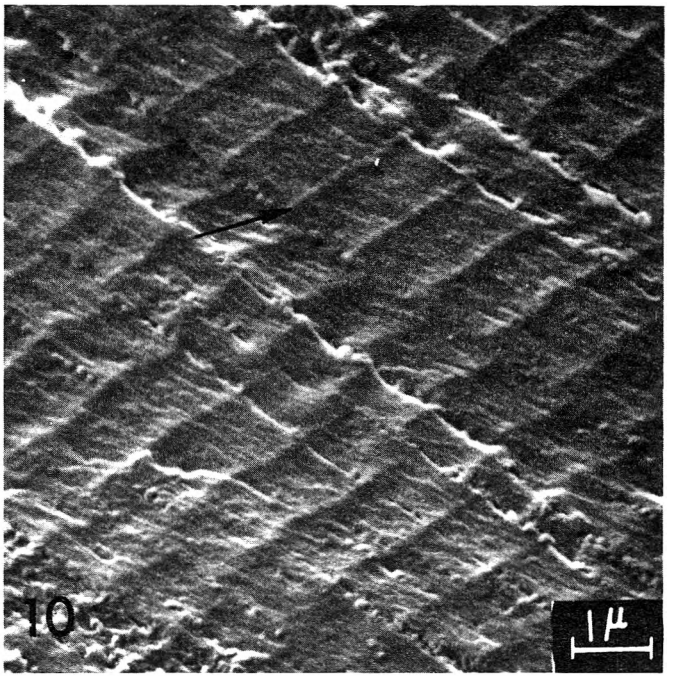

Fig. 10—Longissimus dorsi, cold-shortened, fixed immediately after slaughter, and air dried from ethanol. Surface definition moderate. Sarcomeres are 1.0–1.1μ. Raised structures (arrow) are continuous with sarcomere surface.

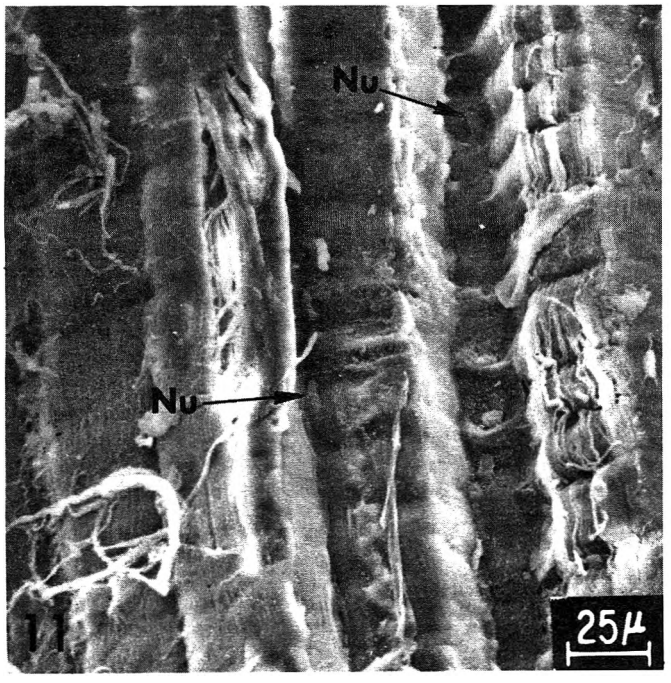

Fig. 11—Fracture longissimus dorsi fixed immediately after slaughter. Fractures occur around fiber surfaces. Numerous nuclei (Nu) present.

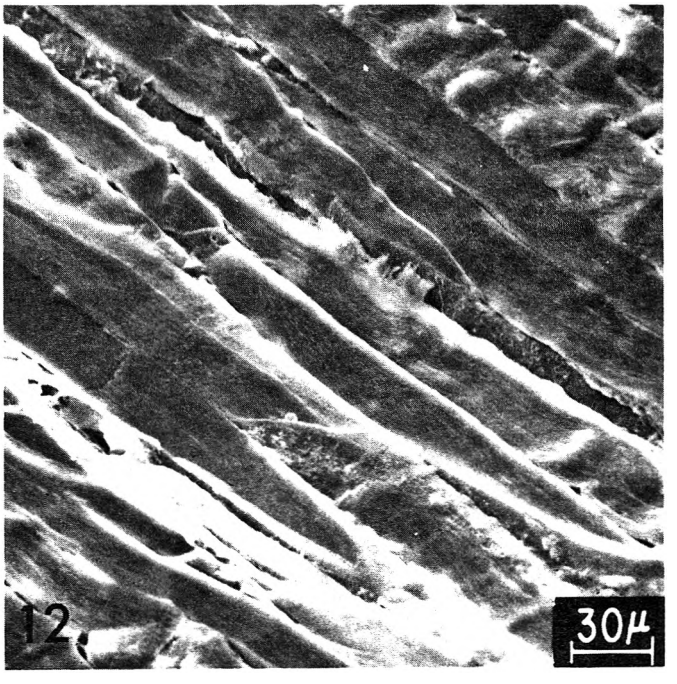

Fig. 12—Fracture through longissimus dorsi, fixed 24 hr after slaughter. Fracture plane is through fibers.

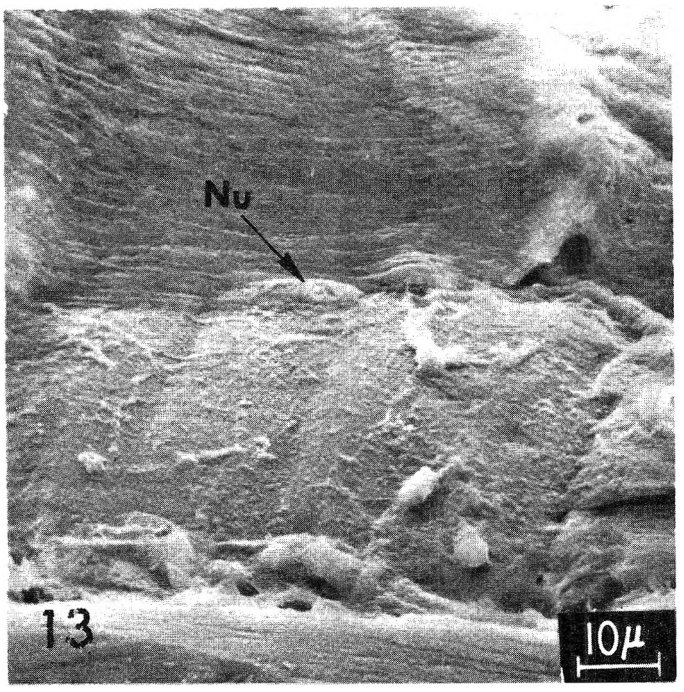

Fig. 13–Longissimus dorsi, fixed 24 hr after slaughter. Top and bottom fibers fractured through; middle fiber sarcolemma exposed. Nucleus (Nu) between fibers.

before or during rigor when muscle is chilled before it has passed fully into rigor (Locker and Hagyard, 1963).

A higher magnification of the surfaces of two cold-shortened fibers is shown in Figure 9. Occasional heavy endomysial collagen fibers (C) lie on top of an extensive network of randomly oriented fibrils, presumably the outer layers of the sarcolemma which encloses the individual muscle fiber. A transverse banding pattern is recognizable beneath the random fibrils.

The myofibril structure of the cold-shortened longissimus is shown in Figure 10. Sarcomere lengths of approximately 1.0–1.1μ give evidence of the high degree of contraction in the tissue. Boundaries between myofibrils are indistinguishable in many places, and sarcomere banding is absent except for raised Z-band–I-band regions (arrow). At this degree of contraction, myosin filaments may buckle against the Z-band, causing bulging. Marsh et al. (1974) suggest that thick filaments actually penetrate the Z-line in highly contracted sarcomeres. This causes overlap of myosin from one sarcomere with actin from the next, a circumstance which could cause distention of the sarcomere in the I-Z area.

The age at which the sample is fixed appears to influence how the samples will fracture. Longissimus dorsi fixed immediately after slaughter exhibited a pronounced tendency to fracture around fiber surfaces (Fig. 11). On the other hand, samples of the same muscle held at 6°C and fixed 24 hr postmortem show fracture planes which shear across the fibers as seen in another low magnification micrograph (Fig. 12). A higher magnification view of the latter (Fig. 13) shows three adjacent fibers; the outer two are fractured through while the sarcolemma is exposed on the center fiber.

The presence of numerous nuclei (Nu) is evident in the freshly fixed samples (both fixed at slaughter and at 24 hr postmortem). These are readily apparent in Figures 11 and 13 as oblong bodies approximately 10μ in length lying along the boundaries between muscle fibers. Nuclei were not found in aged semitendinosus samples.

Sarcomere lengths: effects of fixation and cold shortening

An investigation was carried out to determine if and when shrinkage was occurring in sample preparation. Estimates of sarcomere lengths of unfixed and fixed tissue were made with a compound light microscope on thick muscle sections of commercially-aged semitendinosus suspended in 0.1M phosphate buffer, pH 6.0. Sarcomeres were counted and measured before fixation, after glutaraldehyde fixation, and after osmium postfixation. Unfixed tissue measured 2.5 μ/sarcomere. Each fixation step contributed a 4% reduction in sarcomere length. Solvent dehydration resulted in further shrinkage, easily detected by eye when 2 mm^3 blocks of tissue, glutaraldehyde and osmium-fixed, were taken through the solvent series. Shrinkage was not appreciable until the specimen was immersed in 85% ethanol. When dehydration was continued through the series to higher ethanol concentrations, additional shrinkage did not appear to occur. Even with more steps in the dehydration series (8–10 concentrations; 10 min each) shrinkage still occurred at 85% solvent, without any apparent difference between ethanol and acetone as the solvent. The series finally adopted for general use was 70, 95, 95, 100, 100% ethanol for 5–10 min each depending upon sample size. Drying techniques introduced additional shrinkage; this was kept to a minimum by using small samples. Final sarcomere lengths of the semitendinosus as determined by SEM were approximately 1.9μ.

Cold shortening produced the most drastic shrinkage observed. In the freshly prepared longissimus dorsi, sarcomere lengths were as short at 1.0μ.

CONCLUSION

THE MOST IMPORTANT consideration in obtaining adequate micrographs from muscle tissue was careful preparation of specimens. Fixation was essential. Freeze fracture from ethanol proved to be the most dependable method and produced surfaces untouched by knife or blade. Each drying technique had its good and bad aspects when applied to muscle tissue. Freeze drying and air drying from alcohol produced some shrinkage, but the morphology was crisp and produced good micrographs. Critical point drying produced less shrinkage, but the surface when observed by SEM usually appeared abraded and grainy. In all cases, drying of smaller samples (2 mm or less on a side) produced the best results. It was also necessary to ground the specimens very carefully with silver paint and to coat with metal in the evaporator before observation with the microscope because dried muscle is very porous and charges badly when inadequately grounded. Small samples were advantageous here also.

Most tissue samples which were fractured cleaved through fibers, with the exception of longissimus fixed immediately after slaughter, which tended to cleave around fibers. Commercially-aged semitendinosus had well defined myofibril surface definition. Identification of Z, A, I and H bands was made. Structures suggestive of muscle cell triads were found. Freshly slaughtered longissimus myofibrils were observed in the cold-shortened condition; they were highly contracted and showed well defined contraction waves. Freshly prepared longissimus displayed many nuclei arranged longitudinally along the boundaries of the fibers.

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Ms received 8/25/75; revised 11/22/75; accepted 11/24/75.

Research supported in part by the U.S. Army Natick Development Center Project No. AMXRED 73-161.

Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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PATHWAYS OF FORMATION OF N-NITROSOPYRROLIDINE IN FRIED BACON

ABSTRACT

Pathways of formation of N-nitrosopyrrolidine (NO-Pyr) in fried bacon have been studied. In the range 100–150°C with a heating time of 10 min, amounts of NO-Pyr formed from free proline via pyrrolidine (Pyr) were almost the same as those formed via N-nitrosoproline (NO-Pro). On the contrary, at 175°C or above, the yield of NO-Pyr via Pyr was greater than that formed via NO-Pro. In bacon, 80–90% of NO-Pyr disappeared when it was brought to 200°C within 10 min, while NO-Pyr in vitro was fairly stable to heating, with only a 2–5% loss under the same heating conditions.

INTRODUCTION

MUCH ATTENTION has been paid to the possible formation of carcinogenic N-nitroso compounds in the human environment, particularly in foods. N-nitrosopyrrolidine (NO-Pyr) has been found in approximately 80% of bacon samples tested after frying (Crosby et al., 1972; Fazio et al., 1973; Sen et al., 1973). This is a matter of concern from the public health standpoint since NO-Pyr has been reported to be a powerful carcinogen in some experimental animals (Druckrey et al., 1967; Greenblatt and Lijinsky, 1972a, b; Greenblatt et al., 1973). Crosby et al. (1972) reported that preliminary studies on four different brands of bacon showed that NO-Pyr was formed when the bacon was cooked in a conventional manner but was not present in raw bacon. This discovery was soon followed by research on the mechanism of NO-Pyr formation in fried bacon, and recently, several papers have been published concerning the precursors of NO-Pyr. Bills et al. (1973) have reported that NO-Pyr was produced from N-nitrosoproline (NO-Pro), pyrrolidine (Pyr), spermidine, proline (Pro) and putrescine in yields of 2.6, 1.0, 1.0, 0.4 and 0.04% of their respective theoretical values. No-Pyr could be formed by the nitrosation of Pyr, which may arise from the cyclization of putrescine or from the decarboxylation of Pro. Another possible pathway involves prior nitrosation of Pro followed by decarboxylation to yield NO-Pyr. Recently, the decarboxylation of NO-Pro has been reported (Bills et al., 1973; Sen et al., 1973; Huxel et al., 1974; Fiddler et al., 1974; Pensabene et al., 1974; Gray and Dugan, 1975; Kushnir et al., 1975); however, most of these studies have been conducted in model systems simulating the pan-frying of bacon, under the dry state or by adding the test compounds to either silicone or Wesson oil.

The present study was designed (1) to conduct a commercial bacon survey, with special reference to concentrations of residual nitrite (NO_2^-), Pro, Pyr, NO-Pro and NO-Pyr in various brands of bacon collected in areas of Tokyo and Kanagawa Prefecture, with analyses of bacon done before and after frying; (2) to conduct a quantitative evaluation of the possible pathways of the formation of NO-Pyr from NO-Pro, Pro or Pyr occurring in the actual ground bacon samples at elevated temperatures; and (3) to determine the stability of NO-Pyr in bacon against high temperature heating.

EXPERIMENTAL

Materials

Five different brands of bacon from commercial sources were obtained in areas of Tokyo and Kanagawa Prefecture.

Heating conditions

In order to determine the rate of nitrosation, either Pro (1 mmole) or Pyr (1 mmole) and an equal amount of nitrite ($NaNO_2$) were added to 50g of well-ground bacon sample in a 200 ml beaker, and control preparations were bacon alone or bacon plus nitrite (1 mmole). In order to determine the rate of decarboxylation, either NO-Pro (1 mmole) or Pro (1 mmole) was added to the bacon sample, and control preparations were bacon alone. The beaker was immersed in an oil bath, and the temperature of the contents of the beaker with occasional stirring was brought to 100°, 125°, 150°, 175°, 200° or 225°C within 10 min. This heating system simulated the actual pan-frying of bacon.

Reagents

All reagents and solvent used in the present study were analytical grade. Pro and Pyr were purchased from Wako Pure Chemicals & Co., Tokyo. NO-Pyr was synthesized from Pyr according to the Uno method (Uno and Yamamoto, 1966), and NO-Pro was synthesized from Pro according to the Sander method (1967). The purity of these synthesized compounds was checked by thin-layer chromatography and gas chromatography (GLC), and no detectable impurities were found.

Analytical procedures

NO-Pyr. Determination of NO-Pyr in bacon samples was performed according to the Kawabata et al. method (1974a). The scheme of analysis is shown in Figure 1.

Pyr. Pyr in the sample was determined by GLC after conversion of the compound with $NaNO_2$ to nitrosated Pyr (Kawabata et al., 1973a). The scheme of analysis is shown in Figure 2.

NO-Pro. NO-Pro was determined by GLC after conversion of the compound with diazomethane to NO-Pro methylester (Kawabata et al., 1974b; Ishibashi et al., 1975). The scheme of analysis is illustrated in Figure 3.

The identities of NO-Pyr and methylated NO-Pro were confirmed by gas chromatograph-mass spectrometry.

Free Pro in bacon. A test sample was extracted with 1% picric acid (Hamilton and Van Slyke, 1943), and the extract was passed through an anion exchange resin, Dowex 2 – X 8, in the chloride form, to remove picric acid (Stein and Moore, 1945). Pro in the effluent was determined by an automatic amino acid analyzer, Japan Electron Optics Laboratory Model JLC 6 AH.

NO_2^-. Nitrite residue in bacon was determined according to the method shown in the *Standard Methods for the Examination of Foods*, authorized by the Ministry of Health and Welfare of Japan (1974).

Gas chromatography (GLC)

A gas chromatograph, Shimadzu GC-5 APF equipped with a modified alkali flame ionization detector (AFID; KBr mono-crystal on detector type, Kawabata et al., 1974a) was employed in the present study. For the quantification of NO-Pyr, the test solution in dichloromethane (CH_2Cl_2) purified by the ion-exchange column technique was analyzed on a 3 mm i.d. × 300 cm coiled glass column packed with 15% PEG-20 M on Chromosorb WAW (60–80 mesh), and for the determination of methylated NO-Pro, analysis was made on the same size of glass column packed with 15% DEGS on Shimalite W (60–80 mesh). Analytical conditions for GLC were the same as reported previously (Kawabata et al., 1973a, b; 1974a, b).

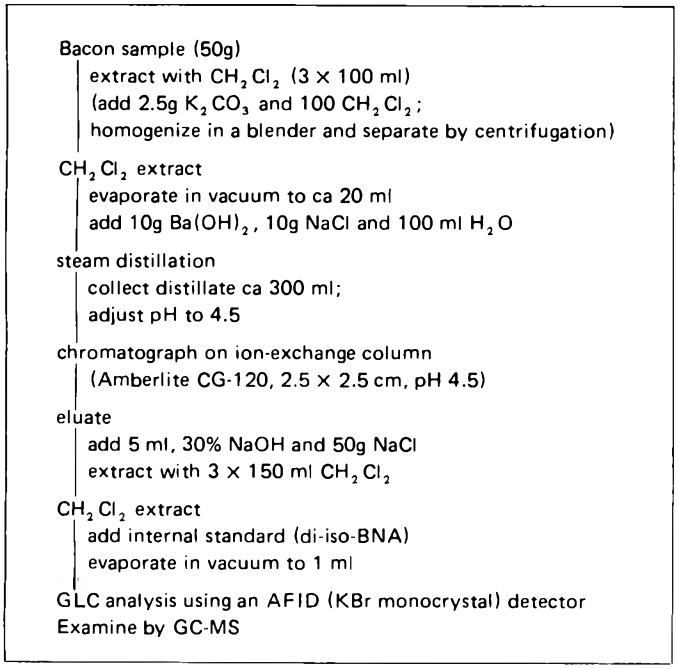

Fig. 1—Procedures for gas chromatographic determination of N-nitrosopyrrolidine (Kawabata et al., 1974a).

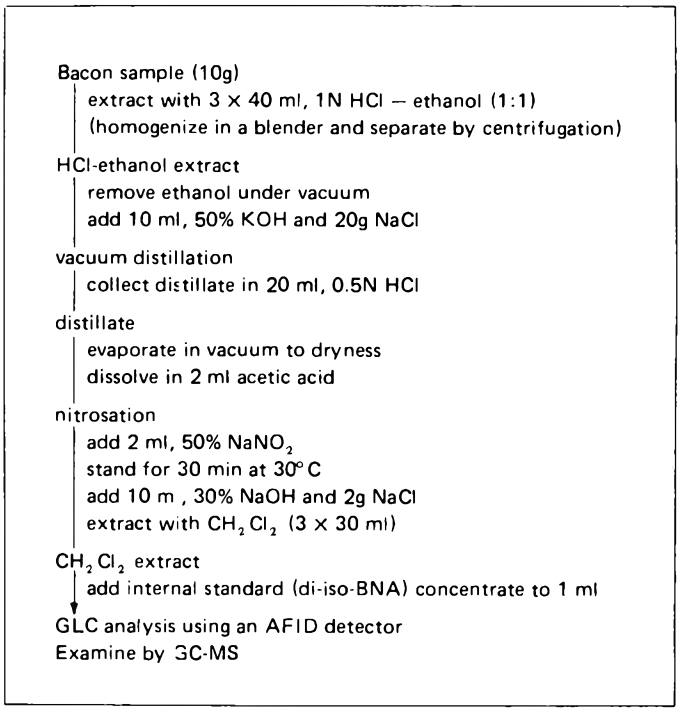

Fig. 2—Procedures for gas chromatographic determination of nitrosated-pyrrolidine (Kawabata et al., 1973a).

Gas chromatograph-mass spectrometric analysis

A gas chromatograph-mass spectrometer (GC-MS), Shimadzu-LKB 9000 was employed for the identification of N-nitroso compounds, and the analytical conditions for GC-MS were the same as reported previously (Kawabata et al., 1974a). The GC-MS fragments applied for the identification of NO-Pyr were m/e 100, 43, 41 and 30, and for the NO-Pro methylester, m/e 158, 128, 99, 69 and 68, respectively.

RESULTS & DISCUSSION

Survey of commercial bacon in Japan

The amounts of NO_2^-, free Pro, Pyr, NO-Pro and NO-Pyr in raw and fried bacon samples of five different brands were analyzed, and the results obtained are shown in Table 1. It was found that NO_2^- residues in the raw bacon samples ranged from 0.6–14 mg/kg. In Japan, the permissible residual level of NO_2^- for ham, sausage and bacon is limited to less than 70 mg/kg, and for fish ham and sausage, to less than 50 mg/kg. These figures are much less than the permissible levels in European countries and the United States. It was also found that NO_2^- residues in raw bacon samples completely disappeared upon frying.

As to the precursor of NO-Pyr in fried bacon, Pensabene et al. (1974) have presumed that NO-Pro could be decarboxylated to form NO-Pyr at higher temperatures. Recently, Kushnir et al. (1975) have reported that NO-Pro was present in raw bacon though the levels were as low as 0.38–1.18 mg/kg. In the present survey, however, no appreciable amount of NO-Pro could be detected in the samples produced in Japan (the minimum detectable limit for NO-Pro was less than 0.01 mg/kg). On the contrary, as shown in Table 1, NO-Pyr was present ranging in concentration from 0.004–0.41 mg/kg in the Japanese bacon when these were cooked at 200°C for 10 min. These facts strongly suggest that the precursor of NO-Pyr in raw bacon is not limited to NO-Pro.

The amounts of free Pro contained in raw bacon ranged

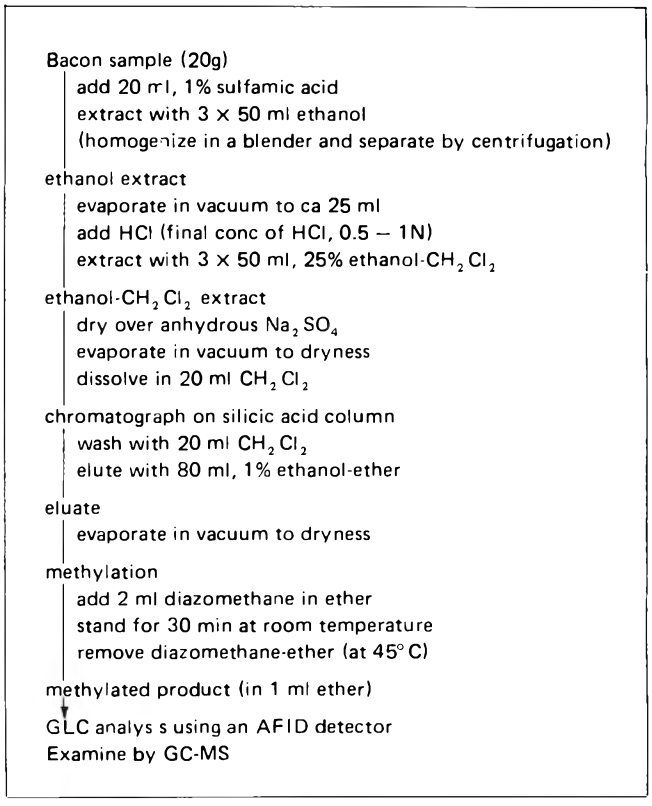

Fig. 3—Procedures for gas chromatographic determination of methylated-N-nitrosoproline (Kawabata et al., 1974b).

from 25–72 mg/kg, and these decreased slightly after the frying process. Concentrations of Pyr in raw bacon were as low as 0.03–0.04 mg/kg. Lijinsky and Epstein (1970) have speculated that pyrolysis of protein and cooking of protein foods may produce free amino acids such as Pro, arginine and hydroxyproline. Fujimaki et al. (1972) have reported that Pyr was formed from L-alanine and D-glucose when heated at 104°C for 16 hr under air. We have found that significant amounts of free Pro were originally present in raw bacon, but the concentration of Pyr was fairly low. Pyr can be formed from Pro by decarboxylation occurring at high temperatures, and the Pyr may be one of the precursors of NO-Pyr in fried bacon. As described previously, no NO-Pro was found in either raw or fried bacon; in addition, no NO-Pyr was determined in raw bacon, while the compound was apparently detected in fried bacon though the concentrations were in mere trace amounts.

Pathways of formation of NO-Pyr

Sen et al. (1973) have suggested two possible pathways of formation of NO-Pyr in fried bacon: (A) formation of NO-Pro from Pro and nitrite, and subsequent decarboxylation to NO-Pyr, or (B) formation of NO-Pyr by direct interaction of Pyr which could be derived from Pro or putrescine and nitrite. In order to test Sen's hypothesis, we made the following experiments:

Formation of NO-Pyr from Pro via Pyr in bacon. The pathway can be expressed as follows:

$$\text{Pro} \xrightarrow{-CO_2} \text{Pyr} \xrightarrow{+NO_2^-} \text{NO-Pyr} \qquad \text{(A)}$$

In the first experiment, 50-g samples of well-ground bacon containing 1 mmole of Pro was brought to 100°, 125°, 150°, 175°, 200°, or 225° within 10 min, followed by determination of any Pyr which might have been formed from Pro by decarboxylation. The results are shown in Table 2. It has become clear that the rate of Pyr formation increased with an increase in temperature, and the maximum yield of Pyr was obtained with heating up to 200°C. In the second experiment, Pyr (1 mmole) and nitrite (1 mmole) were added to bacon, which was then heated as in the first experiment, and the resulting NO-Pyr was determined. The results obtained are shown in Table 3, indicating that NO-Pyr was formed by nitrosation of Pyr, and the maximum yield of NO-Pyr was obtained with heating up to 175°C.

Table 1—Concentrations of nitrite, L-proline, pyrrolidine, nitrosoproline and nitrosopyrrolidine in raw and fried bacon[a]

Brand	Sample Condition	NO_2^- (mg/kg)	Pro (mg/kg)	Pyr (mg/kg)	NO-Pro (mg/kg)	NO-Pyr (mg/kg)
A	raw	1.7	28.0	0.03	N.D.	N.D.
	fried	N.D.	25.0	1.09	N.D.	0.004
B	raw	1.1	30.0	0.04	N.D.	N.D.
	fried	N.D.	27.5	1.15	N.D.	0.007
C	raw	3.6	48.9	0.03	N.D.	N.D.
	fried	N.D.	42.2	1.06	N.D.	0.010
D	raw	0.6	29.8	0.11	N.D.	N.D.
	fried	N.D.	27.6	2.14	N.D.	0.004
E	raw	13.8	72.0	0.03	N.D.	N.D.
	fried	N.D.	61.7	1.03	N.D.	0.041

[a] Minimum detectable limits: NO_2^-, 0.5 mg/kg; NO-Pro, 0.01 mg/kg; NO-Pyr, 0.001 mg/kg. The bacon samples were purchased in areas of Tokyo and Kanagawa Prefecture. Frying condition: bacon samples were brought to 200° C within about 10 min.

Table 2—Formation of pyrrolidine from L-proline in fried bacon[a]

Heating temp (° C)	Pyr formed (mg)	(control)	Yield (%)
100°	0.02[b]	(<0.001)	0.03
125°	0.17	(0.007)	0.24
150°	0.21	(0.009)	0.29
175°	0.90	(0.04)	1.27
200°	1.78	(0.09)	2.50
225°	0.51	(0.02)	0.72

[a] Test preparations: 1 mmole (115 mg) of Pro was added to 50g of raw ground bacon. Controls: bacon without added Pro.
[b] The amounts of Pyr formed in the controls were subtracted from these figures.

Table 3—Formation of nitrosopyrrolidine from pyrrolidine in fried bacon[a]

Heating temp (° C)	NO-Pyr formed (mg)	(control A)	(control B)	Yield (%)
100°	0.19[b]	(<0.001)	(<0.001)	0.19
125°	1.65	(<0.001)	(<0.001)	1.65
150°	1.83	(<0.001)	(<0.001)	1.83
175°	2.08	(<0.001)	(0.001)	2.08
200°	1.93	(<0.001)	(0.001)	1.93
225°	1.52	(<0.001)	(<0.001)	1.52

[a] Test preparations: 1 mmole (71 mg) of Pyr and 1 mmole of $NaNO_2$ were added to raw ground bacon. Controls: (A) bacon alone; (B) 1 mmole of $NaNO_2$ was added to bacon.
[b] The amounts of NO-Pyr formed in the controls were almost negligible.

Table 4—Formation of nitrosoproline from proline in fried bacon[a]

Heating temp (° C)	NO-Pro formed (mg)	(control)	Yield (%)
100°	2.00	(0.04)	1.40
125°	5.82	(0.14)	4.07
150°	5.43	(0.13)	3.80
175°	5.21	(0.09)	3.64
200°	0.97	(0.02)	0.68
225°	0.01	(<0.01)	0.01

[a] Test preparations: 1 mmole of Pro and 1 mmole of $NaNO_2$ were added to 50g of raw bacon. Controls: 1 mmole of $NaNO_2$ was added to bacon.
[b] The amounts of NO-Pro formed in the controls were subtracted from these values.

Formation of NO-Pyr from Pro via NO-Pro in bacon. The pathway can be expressed as follows:

$$\text{Pro} \xrightarrow{+\,NO_2^-} \text{NO-Pro} \xrightarrow{-\,CO_2} \text{NO-Pyr} \qquad \text{(B)}$$

In the third experiment, Pro (1 mmole) and $NaNO_2$ (1 mmole) were added to bacon, and the samples were brought to 100–225°C within 10 min, and then analyzed to determine NO-Pro. The results are shown in Table 4. It was found that nitrosation of Pro occurred in the range 100–175°C, and the maximum yield of NO-Pro was obtained at 125°C. It is to be noted that the formation of NO-Pro apparently decreased at 200°C or above.

In the fourth experiment, NO-Pro (1 mmole) was added to bacon, which was heated under the same conditions as in the first three experiments. The heated samples were analyzed to determine any NO-Pyr which might have been formed from NO-Pro by decarboxylation, and the results are shown in Table 5. It was found that the amounts of NO-Pyr increased with an increase in temperature, and the maximum formation of NO-Pyr was observed at 200°C. It is to be noted that almost no appreciable amount of NO-Pyr was formed at 100°C.

Heat stability of NO-Pyr

As can be seen in Table 5, the amount of NO-Pyr formed at 225°C was apparently lower than that obtained at 200°C. Thus the following experiments were conducted to test the heat stability or the rate of disappearance of NO-Pyr in bacon. Bacon samples containing Pro (1 mmole) and $NaNO_2$ (1 mmole) were brought to 100–225°C within 10 min, and an equal number of samples were brought to the same temperature for a heat-up time of 10 min and held at this temperature for an additional 5 min, followed by the determination of NO-Pyr in all samples. The results are shown in Figure 4. In the samples which had been heated at 200°C or above, marked differences in the amounts of NO-Pyr could be observed between those obtained with a heat-up time of 10 min and those with a heating time of 15 min. The longer the heating time, the lower was the amount of NO-Pyr detected, indicating that at temperatures above 200°C, the rate of disappearance of NO-Pyr may exceed its formation. On the contrary, at 175°C or lower, the amount of NO-Pyr increased with an elongation of the heating time.

Next, the loss of NO-Pyr in vitro (1 mmole of NO-Pyr was distributed in test tubes), and in bacon (1 mmole of NO-Pyr was added to bacon) at 200°C were compared with each other. The results are shown in Table 6. In bacon, about 80–90% of NO-Pyr disappeared when it was brought to 200°C within 10 min, while in vitro, NO-Pyr was found to be fairly heat-stable with a loss rate as low as 2–5%. These results suggest that the retention of NO-Pyr in fried bacon is not only affected by the heating temperature, but also by the presence of certain substance(s) which may associate with the loss of NO-Pyr at higher temperatures. This point, however, needs further clarification.

Relationship between heating temperature and the possible pathways of NO-Pyr in bacon

As shown in Tables 2 to 5, decarboxylation of Pro to give Pyr occurred in the range 175–225°C, and the yields of Pyr increased with an increase in temperature. The maximum formation of Pyr was found to occur at 200°C, while formation was apparently retarded below 150°C. Nitrosation of Pro proceeded at temperatures below 175°C, and the maximum formation of NO-Pro was observed at 125°C. Both nitrosation of Pyr and decarboxylation of NO-Pro actively proceeded at temperatures above 175°C.

Table 7 (the summarized data obtained from Tables 2 to 5) indicates the relationship between the heating temperature and

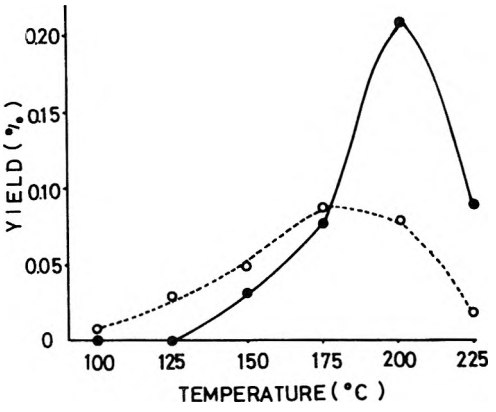

Fig. 4—Effect of heating time on the formation of NO-Pyr from Pro and $NaNO_2$ in bacon at different heating temperatures. ●—●: heating time of 10 min; ○----○: heating time of 10 min plus 5 min.

Table 5—Formation of nitrosopyrrolidine from nitrosoproline in fried bacon

Heating temp (°C)	NO-Pyr formed[a] (mg)	Yield (%)
100°	0.01	0.01
125°	0.01	0.01
150°	0.26	0.26
175°	1.15	1.15
200°	2.53	2.53
225°	0.89	0.89

[a] 1 mmole of NO-Pro was added to raw bacon.

Table 6—Disappearance of nitrosopyrrolidine occurring in preparations heated at 200° C for 10 min

Disappearance rate (%) in vitro		Disappearance rate (%) in vitro[a]	in bacon[b]
2.3,	3.5	87.0,	90.2
2.1,	5.0	82.3,	92.7

[a] 1 mmole of NO-Pyr was heated in a test tube.
[b] 1 mmole of NO-Pyr was added to raw bacon.

Table 7—Comparison of yields of nitrosopyrrolidine formation between those formed via pathway A and B at different temperatures

Heating temp (°C)	NO-Pyr formed Pathway A via Pyr (%)	Pathway B via NO-Pro (%)
100	0.01	0.01
125	0.01	0.01
150	0.01	0.01
175	0.04	0.03
200	0.05	0.02
225	0.01	0.01

the formation of NO-Pyr in fried bacon. In the range 100–150°C, nitrosation of Pro actively proceeds, whereas decarboxylation of NO-Pro to give NO-Pyr is markedly retarded (pathway A). In the same temperature range, decarboxylation of Pro occurs very slowly, while nitrosation of Pyr takes place readily (pathway B). Therefore, reactions involving both pathways A and B may possibly occur simultaneously to yield NO-Pyr although the yields may be mere trace amounts.

On the contrary, the most deeply influencing temperature on the formation of NO-Pyr in fried bacon was found to be from 175–200°C. The amount of NO-Pyr formed from Pro via Pyr (pathway A) was apparently higher than that formed via NO-Pro (pathway B). The pathway A is the main route for the formation of NO-Pyr in bacon when it is fried at 175°C or above; thus decarboxylation of free Pro to yield Pyr actively proceeds at first, followed by nitrosation of Pyr to form NO-Pyr.

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Ms received 9/9/75; revised 11/17/75; accepted 11/24/75.

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EFFECT OF STORAGE ON THE CONCENTRATION OF PROLINE AND OTHER FREE AMINO ACIDS IN PORK BELLIES

ABSTRACT

Proline is a potential precursor in the formation of N-nitrosopyrrolidine, a carcinogen that has been detected in fried bacon. The concentration of free proline and 11 additional amino acids were measured on the first and eighth day after slaughter and storage at 2°C. The free amino acids (proline, alanine, glycine, isoleucine, leucine, methionine, phenylalanine, tyrosine, valine, glutamic acid, cystine, aspartic acid) were determined on separated lean tissue, on the adipose tissue, and on samples of intact green pork bellies. The concentration of most of the amino acids increased with storage. Proline in the intact pork bellies increased approximately 52% after 1 wk of storage. Over the same period, free proline in the lean and in the adipose tissues increased 50 and 90% respectively. Effects of extended storage (28 days) on the concentration of free proline in lean tissue was also determined.

INTRODUCTION

IN 1956, MAGEE AND BARNES reported on the carcinogenicity of N-nitrosodimethylamine in rats. In a few years it became evident that nitrosamines as a class are potent carcinogens in test animals. The possibility exists, although unproven at present, that humans may also be affected by these compounds.

Nitrosamines are formed by the reaction of nitrite with amines, principally secondary amines. Their presence has been reported in numerous foods such as fish, cheeses, and cured meats (Crosby et al., 1972). Of the ten or more possible volatile nitrosamines actively being analyzed for in foods, dimethylnitrosamine, nitrosopyrrolidine, and nitrosopiperidine are the three found most frequently and in the highest concentrations.

While most nitrosamines in cured meat products are not found on a consistent basis, nitrosopyrrolidine has been found consistently in fried bacon (Crosby et al., 1972; Sen et al., 1973; Fazio et al., 1973; Pensabene et al., 1974). Nitrosopyrrolidine, however, has not been detected in uncooked bacon. In the last 2 yr a great deal of effort has been directed toward elucidating the pathway leading to the formation of nitrosopyrrolidine in fried bacon. In 1970, Lijinsky and Epstein suggested that secondary amines could be formed as foods are cooked. They postulated that the cooking of tissue protein could produce free amino acids such as proline and hydroxyproline, and that the secondary amine, pyrrolidine, could be formed by heating the diamine putrescine; which is known to be present in meat and in fish. They also postulated that proline ingested in foods could be nitrosated in the stomach to nitrosoproline, and then enzymatically decarboxylated to nitrosopyrrolidine by bacteria in the duodenum and the small intestine. Bills et al. (1973) demonstrated in model systems that nitrosopyrrolidine could be produced from such precursors as putrescine, proline, and nitrosoproline, when subjected to conditions similar to those encountered on frying bacon. Kushnir et al. (1975) isolated and identified nitrosoproline in uncooked bacon and suggested this is a precursor for nitrosopyrrolidine. Gray and Dugan (1975) recently demonstrated nitrosopyrrolidine formation by heating nitrite with ham connective tissue or collagen; both types of preparations contain relatively large quantities of bound proline and hydroxyproline. It seems evident that proline, a natural component of meat, could be a precursor of nitrosopyrrolidine.

Several investigators (Bowers, 1969; Usborne et al., 1968) in their research on porcine muscle quality and flavor, have determined the presence of free amino acids. However, the studies were limited to examination of the composition of the longissimus muscle.

The object of the study reported here was to determine the concentration of free proline and other amino acids in the lean and adipose tissues of green pork bellies before processing into bacon and to note the changes that occur during the first week of storage, a reasonable holding period for green bellies prior to curing.

EXPERIMENTAL

Methods and materials

Pork bellies were obtained from a commercial processor approximately 24 hr after slaughter. Six bellies were skinned, quartered, and sliced into small pieces. Random samples were removed, mixed, and 100g used for each analysis. This represented the intact tissue. Other portions of these bellies were separated into lean and adipose tissue; the tissues were cut into small segments and mixed in order to achieve homogeneity. The composition of bellies was approximately 62% adipose and 38% lean tissues. The flow chart of the procedure for extraction of proline is shown in Figure 1. 100g of tissue was macerated

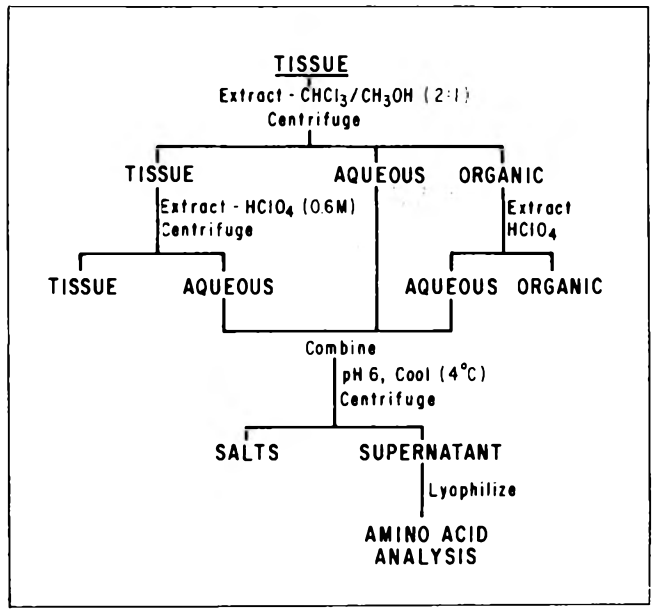

Fig. 1—Flow chart of procedure for extraction of proline.

and extracted in a blendor (Waring, Model 91-262) with 100 ml Folch's reagent (2/1 chloroform/methanol) at high speed for 1 min. The slurry was centrifuged and the three layers [tissue, chloroform (organic) layer, and upper aqueous phases] were separated. The tissue residue was reextracted twice with 100 ml Folch's reagent and the supernatants combined. The combined chloroform layers were extracted three times with 100 ml of 0.6M perchloric acid. The perchloric acid extracts were pooled. The residual tissue was extracted in a blendor with perchloric acid and centrifuged. The layers were separated and the tissue extracted twice more. All perchloric acid extracts and the initial aqueous phases were combined and adjusted to pH 6 with KOH and cooled. The precipitated potassium perchlorate was removed by centrifugation. The precipitate was washed with ice cold deionized water and the washings were added to the supernatant. The supernatant was concentrated by freeze drying and redissolved in 10 ml of sodium citrate buffer, pH 2.2. The acidic and neutral amino acids were separated by passing the extract through a column packed with Spherix XX8-60-0 resin using an accelerated system developed by Spackman et al. (1958). The instrument used was a Phoenix Precision Instrument Company amino acid analyzer model M-7800.

To study the effect of storage, green bellies were kept in a refrigerator at 2°C for 1 wk and then assayed as described. A separate storage study extended over a 4-wk period was also conducted. Two bellies were used for this study and the determinations done in duplicate.

RESULTS & DISCUSSION

THE CONCENTRATIONS of free proline found in the tissues from six fresh (green) bellies are shown in Table 1. The pork was stored for 8 days and the free amino acids were determined on the first and eighth day. The average proline content of intact tissue and lean tissue (adipose removed) was 14.9 μM and 23.9 μM per 100g of wet tissue, respectively. The concentrations of free proline in the intact and lean tissues both increased approximately 50% (51.6% and 47.6%) after storage for 1 wk. Adipose tissue contained less than one-half the concentration of free proline present in the intact tissue. After 1 wk storage, however, free proline in the adipose tissue increased 96%. The increase in free proline due to proteolysis during postmortem storage is expected, but the fact that the rate of increase is twice as great in the adipose tissue as in lean is not expected. No explanation can be offered at this time.

Based on the concentration of proline present in adipose and lean tissues, and with the knowledge of the ratio of these tissues in the whole belly, the concentration of proline in the intact belly can be determined. Table 2 shows that these values approximate the concentrations actually found.

The average concentration of the other acidic and neutral amino acids measured in this study are summarized in Table 3. Alanine, glycine, isoleucine, leucine, methionine, phenylalanine, tyrosine and valine increase in all tissues after a week of storage. The glutamic acid level increased in the intact and lean tissue after 8 days but remained unchanged in the fatty tissue. The concentration of cystine did not appear to change, and aspartic acid showed a decrease in the intact and lean tissue after 1 wk.

The observation that a greater degree of proteolysis takes place in adipose tissue on storage, as is the case with proline, was also noted in nine of the 11 other amino acids measured. Only glycine and glutamic acid demonstrate a greater relative percentage increase in the lean tissue.

The effects of extended storage at 2°C on the concentration of free proline in the lean tissue of green bellies is given in Table 4. Proline concentration was determined over a 4-wk period. The average concentration of proline increased from 26 μM per 100g on the first day to 80 μM on the 28th day. After 16 days, spoilage was clearly evident from the odor and color of the meat. Macerating this tissue did not yield higher levels of free proline.

Several studies on the conditions required for formation of nitrosamines from amino acids have been performed, including such parameters as pH, temperature, and nitrite. Bills et al. (1973), tested glutamic acid, glutamine and hydroxyproline in

Table 1—Effect of storage at 2°C on the concentration of free proline in pork bellies—intact, lean, and adipose tissues

	Free proline (μM/100g of tissue)					
	Intact		Lean		Adipose	
Sample	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8
1	21.1	30.2	25.8	36.3	5.8	9.4
2	16.4	19.4	14.2	37.2	5.3	15.4
3	12.1	24.9	22.0	30.6	6.7	7.2
4	13.7	14.9	35.3	38.4	5.1	12.1
5	11.7	25.8	18.9	31.6	6.7	10.1
6	14.2	20.4	26.7	37.9	4.4	12.9
Avg	14.9	22.6	23.9	35.3	5.7	11.2

Table 2—Concentration of free proline in intact bellies actually found, as compared to that determined by calculations from lean and adipose tissues

		Free proline (μM/100g of tissue)		
		Adipose	Lean	Intact belly[a]
Day 1 —	Found	5.7	23.9	14.9
	Calculated[b]			12.6
Day 8 —	Found	11.2	35.3	22.6
	Calculated[b]			20.2

[a] Average green belly composition: 62.3% adipose and 37.7% lean
[b] Calculated to composition of intact green belly

Table 3—Effect of storage at 2°C on concentration of free amino acids in pork bellies—intact, lean and adipose tissues

	Conc of free amino acids (μM/100g tissue)[a]					
	Day 1			Day 8		
Amino acid[b]	Intact	Adipose	Lean	Intact	Adipose	Lean
Ala	143	40	244	188	58	320
Gly	109	32	148	132	37	181
Ile	10	3	14	15	9	21
Leu	17	6	23	29	20	35
Met	4	1	6	13	6	21
Phe	8	3	11	14	9	19
Tyr	7	3	11	11	6	14
Val	18	6	28	28	20	33
Glu	27	19	33	50	17	98
Cys	15	5	19	15	7	18
Asp	19	2	55	12	6	17

[a] All values are averages of six samples.
[b] Ala, Alanine; Gly, Glycine; Ile, Isoleucine; Leu, Leucine; Met, Methionine; Phe, Phenylalanine; Tyr, Tyrosine; Val, Valine; Glu, Glutamic Acid; Cys, Cystine; Asp, Aspartic acid

Table 4—Effect of extended storage at 2°C on the concentration of free proline in the lean tissue of pork bellies[a]

	Days postmortem						
	1	2	4	7	14	21	28
Sample	(μM/100g)						
A	27.2	40.0	38.8	51.0	40.8	57.0	90.3
B	25.6	22.8	37.2	43.8	40.8	44.0	71.0
Avg	26.4	31.4	38.0	47.4	40.8	50.5	80.7

[a] All values are averages of duplicate determinations.

an oil-water system, which at 170°C in the presence of sodium nitrite, did not form nitrosopyrrolidine. Ender and Ceh (1971) found that heating leads to the decarboxylation of amino acids to form corresponding amines, but only proline leads to the formation of nitrosopyrrolidine. Glycine, sarcosine (N-methylglycine), and valine produced mainly dimethylnitrosamine, while alanine formed dimethylnitrosamine and diethylnitrosamine. It appears evident that the major amino acid which may be an in vitro precursor in the formation of nitrosopyrrolidine is proline; and that the concentration of free proline increases in both the lean and adipose tissues of green bellies during storage, but at a faster rate in the latter.

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Ms received 11/10/75; revised 1/15/76; accepted 1/20/76.

Reference to brand or firm names does not constitute endorsement by the U.S. Dept. of Agriculture over others of a similar nature not mentioned.

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DENSITY SEPARATION OF PROTEIN AND CARBOHYDRATES IN A NONAQUEOUS SOLVENT SYSTEM

ABSTRACT

The proteins and carbohydrates in wheat flour, soybeans, green peas and dried acid whey were separated on the basis of density in a nonaqueous solvent system. By dispersing finely milled flour in solvent ranging in density from 1.3–1.5 a protein rich fraction can be readily separated from a carbohydrate-rich fraction by centrifugation. The protein fraction from wheat flour contained from 58–81% protein while the carbohydrate fraction contained only from 0.4–3.9% protein. After separation and recombination the baking quality of the wheat flour showed little modification. Similar separations were obtained for the other protein sources tested. Trichlorofluoromethane (Freon 11) mixed with ethanol, acetone, ethyl acetate, or hexane were evaluated as solvent systems; the ethanol-containing mixtures gave significantly better separations than the other solvent systems. Acetic acid and formic acid were not effective for improving separation while addition of sodium hypochlorite did improve the separation of protein and starch.

INTRODUCTION

IMPROVING TECHNIQUES of wheat protein-starch separation have been a challenge of long standing. In early days, the cosmetic properties of starch made it a highly desirable commodity. More recently, there has been worldwide emphasis on proteins. Functional proteins are of particular interest for their nutritional properties and the physical improvements they afford fabricated foods. The unique properties of gluten have, therefore, been more widely recognized, resulting in a related growing interest in the recovery and utilization of gluten and related cereal proteins. Fellers (1973) and Anderson (1967) have recently reviewed the current processes for recovering wheat proteins by aqueous methods. All of these methods can entail water pollution problems in the form of waste water streams. Ideally, a nonaqueous process could avoid water pollution and, for solvents with low specific heats generate substantial energy savings in both drying and solvent recovery operations.

A nonaqueous system for wheat protein-starch separation can be approached in two ways: first, selective solubilization of the protein or the starch by a solvent, or second, a physical separation of the carbohydrate and protein fractions by density differences. Previously, Hess (1954) reported that interstitial protein can be separated from endosperm starch on the basis of density, using a mixture of benzene and carbon tetrachloride. Stevens (1963) employed the same technique to recover protein from air classified material for chemical characterization. Barlow (1973) has also reported efforts to recover protein by similar density separations. In this work, the density separation approach was selected and solvents of intermediate density between the wheat protein and starch were used. For this approach to be practical the solvents used for the nonaqueous separations of food proteins must have the proper density, be nontoxic, and have both a low boiling temperature and low heat of vaporization. For economy reasons, the solvent must also be easily condensed and completely recoverable. The chlorinated-fluorinated hydrocarbons used in this work appear to meet these criteria.

EXPERIMENTAL

Materials

Unbleached flour (12.1% protein) from hard red winter (HRW) wheat (ConAgra-Montana Mills, Oakland, CA) was remilled in a Pillsbury Turbomill to reduce particle size and was used throughout this study unless otherwise specified. Acid whey (13.1% protein), split dried peas (24.4% protein), and one portion of wheat flour were milled overnight in a ball mill to afford finely powdered starting materials. Similarly milled dehulled soybeans (40.2% protein) gave a plastic mass similar to butter. Defatted soy flour (52% protein) was obtained from Central Soya, Chicago, IL.

Fractionation

Typically the fractionation was carried out by suspending 1.0g portions of flour (10.1% moisture) in 100 ml of either hexane-Freon or benzene-carbon tetrachloride at 0°C. The mixtures were blended for 30 sec with a Du More shear-type blender and samples were centrifuged at 0°C in a Sorvall RC2-B refrigerated centrifuge using 40 ml cups in a swinging bucket head for 10 min at 10,000 RCF. The floating protein fraction was carefully decanted into an evaporating dish. Generally about half of the solvent was decanted with the floating portion (protein) and half was left with the precipitated material (carbohydrate). The two fractions were then dried in a hood prior to Kjeldahl-N and moisture determinations.

Acetic acid and sodium hypochlorite were tested as additives to the solvent system to improve the separation of protein from starch granules in flour. One ml of a 5% aqueous solution of these materials was added to 99 ml of Freon + hexane prior to flour addition. Ethanol, acetone, and ethyl acetate were also substituted for hexane to reduce the density of the Freon 11.

Large scale fractionation

Large scale separations were carried out by slurrying 100g of flour in 1,000 ml of Freon 11-hexane (density = 1.425), blending for 30 sec, and centrifuging at 0°C and 10,000 RCF for 10 min in a Sorvall RC2-B centrifuge equipped with a GSA rotor. The floating and precipitated materials were removed and air dried as described above.

Analytical procedures

Density was determined by means of a hydrometer corrected to 0°C. Nitrogen, moisture, and farinograph measurements were carried out using standard AACC methods (1962). Farinograms were obtained by means of a Brabender Farinograph equipped with a 50g bowl. Baking tests were carried out as described by the USDA Agricultural Stabilization and Conservation Service (1971).

RESULTS & DISCUSSION

FOR DENSITY SEPARATION to be most effective, the difference between the densities of the component fractions must be as great as possible. Table 1 shows the effect of the moisture content on the effective densities of wheat protein and starch as calculated from the data of Hess (1954). As the moisture content of the protein and starch increases, the effective densities of both protein and starch decrease. Additionally, and of particular importance is the fact that the differences in density between the protein and carbohydrate fractions decrease with increased moisture content. Since the present work utilizes small differences in density it is important to maximize these differences by maintaining as dry a

system as practical. Unfortunately, preliminary tests with low moisture material indicated that flour moisture levels of 10–12% are required for effective separation of wheat protein and carbohydrate fractions. This moisture level is convenient as it is the level normally obtained for flours which are allowed to equilibrate with the atmosphere.

For density separations to be effective the solvent density must be intermediate between the density of the protein and that of the starch. The desired solvent density for separating flour of 12% moisture would, therefore, be between 1.303 and 1.440 (Table 1). This narrow range substantially limits the availability of suitable solvent systems; however, the physical properties of fluorinated and chlorinated hydrocarbons (Table 2) make them of interest. Of the possible solvents both Freon 12 and Freon 114 offer excellent properties for protein starch separations; unfortunately, their low boiling points make laboratory handling of these solvents somewhat difficult, requiring the use of refrigeration equipment or high pressure apparatus. The current work has been carried out with the higher boiling Freon 11 (CCl_3F) and carbon tetrachloride, which can be used in combination with a lower density solvent to generate mixed solvent systems with the correct density for the desired separation. The relationship between density and solvent composition for hexane-Freon 11 mixtures at two different temperatures is shown in Figure 1. Small amounts of hexane can be mixed with Freon 11 to provide a solvent system of varying density. Additionally, the change in solvent density with temperature control is critical to the success of the protein-carbohydrate separation.

Separations of ball milled wheat flour were carried out in several hexane-Freon 11 and benzene-carbon tetrachloride mixtures of varying densities. The protein-containing layer (float) and carbohydrate (sink) fraction were separated and analyzed for composition. Results of the distribution of protein and carbohydrate fractions versus solvent density are shown in Figure 2. As expected, the percentage of floating material increased with increasing solvent density, particularly above 1.430 for hexane-Freon 11 and above 1.450 in the benzene-carbon tetrachloride solvent system. It can also be seen in the Freon 11-hexane solvent system that the protein content of the floating fraction reaches a maximum of 80% as the solvent density reaches 1.350. Above this solvent density the amount of floating material also increases, but the percent protein in the float drops slowly up to a solvent density of 1.430 and then drops rapidly as more and more carbohydrate material becomes incorporated into the float. Microscopic examination of the isolated materials showed the high protein isolates to contain small starch granules trapped in the protein particles of the floating fraction. At higher solvent densities, larger starch granules were in the protein and eventually in-

Table 1—Effect of moisture on density of wheat protein and starch[a]

Moisture (%)	Effective density		
	Protein	Starch	Difference
0	1.345	1.500	0.155
4	1.331	1.480	0.149
8	1.317	1.460	0.143
12	1.303	1.440	0.137
16	1.290	1.420	0.130

[a] Calculated from data presented by Hess, 1954.

Table 2—Physical properties of fluorinated hydrocarbons

DuPont name	Chemical formula	Density as liquid at 25°C	Boiling point °C	Heat of vaporization
Freon 11	CCl_3F	1.476	23.82	43.10
Freon 12	CCl_2F_2	1.311	−29.79	39.47
Freon 113	CCl_2F-$CClF_2$	1.565	47.57	35.07
Freon 114	$CClF_2$-$CClF_2$	1.456	5.77	32.51
Freon C318	c-C_4F_8	1.500	−5.85	27.77

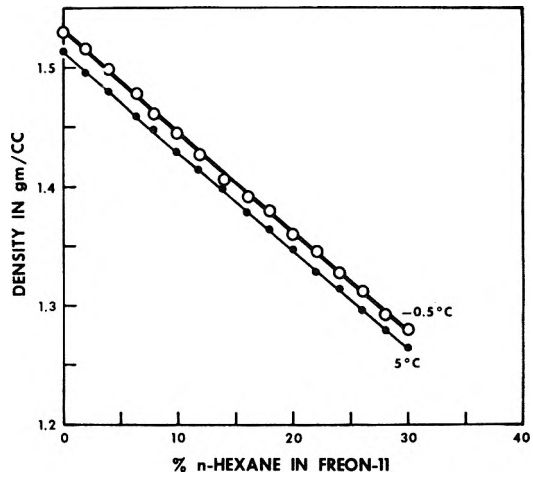

Fig. 1—Densities of hexane:Freon 11 mixtures at two temperatures.

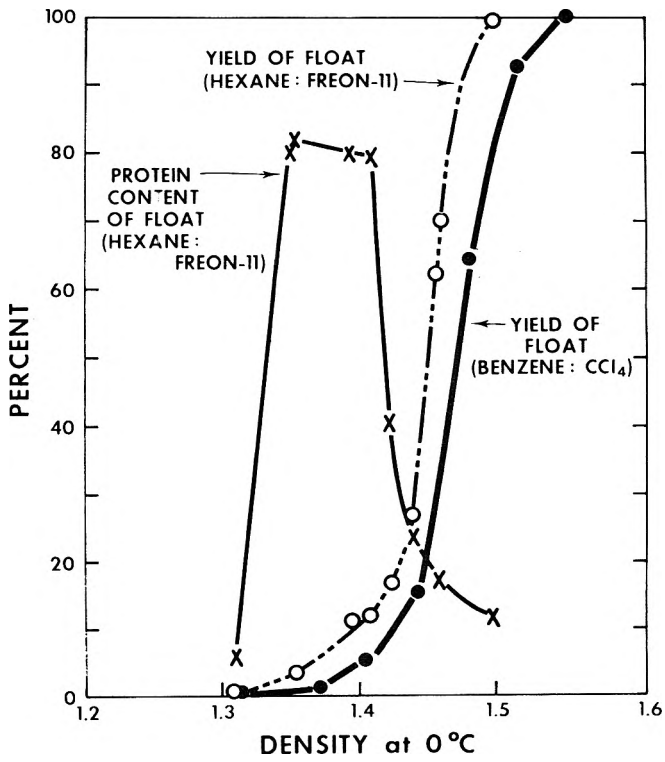

Fig. 2—Effect of solvent and solvent density on the fractionation of ball-milled wheat flour for two solvent systems.

Table 3—Protein recoveries from density separations of HRW flour treated in various ways

Flour treatment[a]	% flour solids in:		Protein content, % mfb		% of total flour protein in float
	Float	Sink	Float	Sink	
Unbleached	13.56	86.44	73.0	2.58	81.8
Ball milled	11.04	88.96	93.6	1.98	85.4
Turbomilled	12.07	87.93	85.3	2.05	85.1
Turbomilled + 1% acetic acid[b]	12.20	87.80	84.7	2.01	85.4
Turbomilled + 1% formic acid[b]	12.25	87.75	84.9	2.03	85.7
Turbomilled + 1% sodium hypochlorite[b]	12.51	87.49	92.7	0.46	95.8

[a]All flours processed were 10.1% moisture and 12.1% protein on a dry basis. The flour-to-solvent ratio was 1:100 (w/v) and the solvent was 14:86 (v/v) hexane:Freon 11 with a density of 1.415 at 0°C.
[b]Added as 1 ml of a 5% aqueous solution to 99 ml of solvent.

dividual starch granules similar to those found in the original flour were found in the floating portions.

Even under optimum conditions it appeared that a better protein-starch separation could be obtained if the samples were either reduced in particle size or something could be added to the solvent system that would break the protein-carbohydrate interaction. Thus, samples of original unbleached, ball milled, and turbomilled flours were separated at a single solvent density (1.415) to determine the effect of particle size on protein-carbohydrate separations. Concomitantly the effects of acetic acid, formic acid, and sodium hypochlorite on the protein-starch separation of turbomilled flour were also evaluated at a solvent density of 1.415. Results of these separations are shown in Table 3. The percent protein in the floating fraction was increased by finer milling, as evidenced by the fact that the percent protein of the float of ball milled flour (93.6%) was greater than that of turbomilled flour (85.3%), which in turn was greater than that of the original unbleached flour (73.0%). The percentage of total flour protein in the float followed the same sequence (85.4%, 85.1% and 81.8%, respectively); however, the increased milling did not enhance the protein yield sufficiently and chemical treatment was evaluated. Table 3 compares the effect of adding acetic acid, formic acid, and sodium hypochlorite to the solvent system on the density separation of turbomilled flour. Acetic and formic acids do not appear to have a significant effect on the protein-carbohydrate separations. Sodium hypochlorite, however, does improve the separation significantly, yielding nearly 95.8% of the protein in the floating fraction. The protein content of the starch fraction is extremely low (0.4%) which suggests that the starch could be of significant use to the food industry. Microscopic examination of the slurries containing sodium hypochlorite showed the starch granules to be much more devoid of surface absorbed protein than the starch granules in slurries of other treatments. It is evident that the sodium hypochlorite caused the protein to separate from the starch, yielding an easily recoverable protein concentrate.

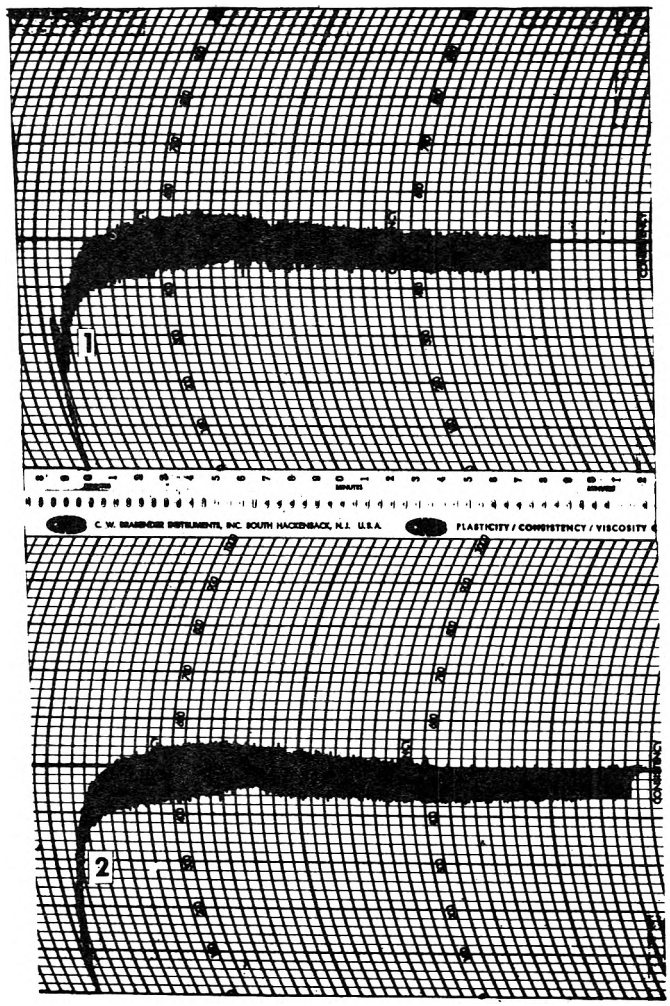

Fig. 3—Farinograph of (1) control flour and (2) solvent fractionated reconstituted flour. Absorptions were 57.2 and 60.2, respectively.

Hexane was initially chosen as the solvent for adjusting the density of Freon 11 because it was a volatile, low density, soluble liquid. Several other low density solvents with slightly different properties were also evaluated as possible co-solvents for protein-carbohydrate density separations. The effects of replacing hexane with ethanol, acetone, and ethylacetate are shown in Table 4. The data show that ethanol-Freon 11 yielded the purest protein (93.7%) in the floating fraction. Absolute ethanol must be used for the separation because 95% ethanol increased the water content of the system, causing agglomeration of protein and entrapment of starch granules. The total protein recovered in the floating fraction, however, was only slightly greater than that recovered with other solvent systems. Hexane, ethyl acetate, and acetone appear to be somewhat poorer solvents in combination with Freon 11 for achieving the separation of protein and carbohydrates in wheat flour.

The effect of nonaqueous solvents on the baking properties of wheat flour were studied by separately isolating the floating

Table 4—Protein recoveries from turbomilled flour when various low density mixed solvents are used in the density separation process

Solvent ratio	Density	% Flour solids in:		Protein content, % mfb		% of total flour protein in float
		Float	Sink	Float	Sink	
Freon 11:hexane (14:86)	1.415	13.57	85.43	73.0	2.6	81.8
Freon 11:ethylacetate (19:81)	1.415	13.41	86.59	78.5	2.1	84.7
Freon 11:ethanol (17:83)	1.415	11.01	88.99	93.7	2.0	85.6
Freon 11:acetone (17:83)	1.415	13.68	86.32	71.9	2.7	81.3

and sinking phases from a large-scale preparation and recombining the two phases. Comparison of the farinograms of the separated and recombined flour and the original flour (Fig. 3) indicated that the absorption of the recombined material increased from 57.2 to 60.2%, indicative of starch damage sustained in the treatment. The farinograms also suggest that after prolonged mixing the recombined flour may be weakened slightly more than the control. This difference, however, is quite small and may be of limited significance. Baking the treated and untreated flours into pup loaves resulted in bread volumes of 735 cc and 690 cc, respectively. Of significance is the fact that nonaqueous separation does not appear to damage the dough forming character of gluten and apparently has only a limited overall effect on flour. This would imply that nonaqueous separations conducted on a large scale could be an efficient method for producing a concentrated protein from wheat with little or no change in its physical properties. Such protein could then be used in applications similar to current uses of vital gluten although more water soluble proteins would be included in the density separated material than in normal water washed vital gluten.

Having established a simple system for separating flour proteins, dried acid whey, soybeans, defatted soy flour, and dry green peas were separated using the devised nonaqueous solvent system. The separations of ball-milled materials using a solvent of 1.415 density are shown in Table 5. The results suggest that the protein-starch separation for all three materials is feasible. The protein content of the peas floating fraction was relatively low but the overall protein recovery was similar to the other materials tested. The purest protein was obtained in the acid whey floating fraction which contained 93.6% protein. For soybeans, the hexane content of the mixed solvent had to be reduced because the fat in the soybeans tended to reduce the density of the solvent. This change in density would become very significant when large amounts of a high fat material are separated in continuous systems. It should be pointed out, however, that such a system then also becomes a convenient single process to recover fat, protein, and carbohydrates from fat containing materials such as oilseeds. It thus appears that nonaqueous separations work on a variety of protein sources requiring only that the material be finely ground to achieve good separation of protein and carbohydrate fractions.

Table 5—Recovery of protein concentrates from soybeans, green peas, and acid whey by density separation in hexane:CCl_3F (14:86) at 0°C. Product-to-solvent ratio was 1:100 w/v

Protein source	Protein content, of float, %, mfb	% of total protein recovered in float
Soybeans	87.2	84.1
Defatted soy flour	87.3	84.3
Dry split peas	63.1	80.7
Whey	93.6	78.5

CONCLUSIONS

LABORATORY EXPERIMENTS have demonstrated that the protein and carbohydrate components of wheat flour, soybeans, green peas, and acid whey can be separated on a density basis in nonaqueous solvent systems. Addition of sodium hypochlorite to the solvent considerably improves the separation of wheat protein from carbohydrates. Baking the recombined components from wheat flour demonstrates that separation of protein and starch can be achieved without serious damage to the functional properties of the protein. Potential advantages of nonaqueous density separation process are: reduced energy requirements, elimination of water pollution problems, and retention of the native functional properties of the protein by elimination of hydration changes and thermal damage as found in conventional drying procedures. Precautions must be taken to prevent solvent losses, and the safety of fluorinated hydrocarbons must be proven beyond a reasonable doubt before any large-scale operation is undertaken.

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Ms received 10/3/75; revised 12/13/75; accepted 12/16/75.

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EFFECT OF ENZYME RATIO AND pH ON THE EFFICIENCY OF AN IMMOBILIZED DUAL CATALYST OF GLUCOSE OXIDASE AND CATALASE

ABSTRACT

Glucose oxidase and catalase were immobilized to a support of Ni-impregnated silica alumina. The efficiency, defined as how well the second enzyme in the reaction sequence utilizes the product of the first enzyme-catalyzed reaction, was determined as a function of the activities of the two enzymes on the support. Catalase efficiency decreased with decreasing catalase activity. At a constant ratio of enzymic activities, efficiency of the dual system decreased with decreasing absolute activities. The dual immobilized system exhibited an optimal pH at 5.5 (optimal pH of glucose oxidase); this was not observed with either a soluble or an immobilized mixed system.

INTRODUCTION

THE POTENTIAL USE of immobilized enzymes in food processing is considerable (Olson and Richardson, 1974). Potentially, the use of multi-enzymic systems in many processing operations may be necessary. Thus, attention must be devoted to this facet of immobilized enzyme research.

Mosbach and Mattiasson (1970) constructed a two-enzyme system of hexokinase and glucose-6-phosphate dehydrogenase immobilized to various organic polymer supports. They demonstrated that there was an increase in the overall reaction of the immobilized enzyme system compared to the soluble system which they suggested was due to the build-up of the intermediate in the microenvironment of the immobilized enzyme. The second enzyme in the system acted on the intermediate before it had time to diffuse away. The same workers (Mattiasson and Mosbach, 1971) later showed that the same phenomenon was observed in a three-enzyme system except that it was more pronounced. A theoretical analysis led Goldman and Katchalski (1971) to predict what Mosbach and Mattiasson had observed, namely that the rate of production of the end product in the first stages of the reaction was markedly higher with an immobilized two-enzyme system than would be predicted for the corresponding homogeneous system.

Srere et al. (1973) demonstrated that an immobilized system may be more efficient compared to a corresponding free enzyme system during the whole course of a reaction and not just at the initial stage as a consequence of shifting the apparent equilibrium conditions because of the build-up of intermediates in the microenvironment of the enzymes. Gestrelius et al. (1972) showed that immobilized enzymes can be affected by pH changes caused by reactions catalyzed by other enzymes with the build-up of acid or base in the microenvironment. Vasil'eva et al. (1969) examined the kinetics of a coupled reaction catalyzed by hexokinase and polynucleotide disphosphorylase in a homogeneous solution and in two nucleoprotein coacervates. In one case, the enzymes were in different phases of the coacervate; in the other they were in the same droplet phase. The latter gave a higher reaction rate than with the same enzymes in homogeneous solution or in the different phases of the coacervate.

Messing (1974) immobilized glucose oxidase and catalase by adsorption within the pores of titania, and observed a remarkably stable enzyme system. Parameters were able to be adjusted to vary loading. He suggested that catalase apparently acts both as a stabilizer and an activator for glucose oxidase within the pores of the titania.

In the work reported here, we have studied the effect on the efficiency of functioning of the dual enzyme system of glucose oxidase and catalase of varying the amounts of the activities of the enzymes immobilized to the support surfaces (Bouin et al., 1975). We also examined the effect of pH on the functioning of this dual enzymic system. The immobilized system of glucose oxidase and catalase has potential use in the production of gluconic acid and in the removal of glucose and/or O_2 from liquid food systems.

EXPERIMENTAL

Materials

Glucose oxidase (GO_x) from *A. niger* (110 I.U./mg) and soluble beef liver catalase (Cat) (105,000 I.U./mg) were purchased from Worthington Biochemical Corp. Bovine serum albumin (BSA) was obtained from Sigma. Enzymes were used without further purification. Glutaraldehyde was a product of Fisher Scientific Co., and glucose was obtained from Matheson, Coleman and Bell. The support used was a silica alumina impregnated with 5% nickel (No. Ni 0901-S; Harshaw Chemical Co., Solon, Ohio). We ground the silica alumina and screened it to a uniform size of 125–149 microns (100–120 mesh). The surface area and pore diameter of the support particles ranged from 0.5–1.0 m^2/g and from 2–40 microns, respectively. All other chemicals used were the purest available commercially.

Method of immobilization

Nickel-impregnated silica alumina particles of uniform diameter were treated with gamma-aminopropyltriethoxysilane (APTES) according to a procedure similar to that used by Weetall and Hersh (1970) to produce a functional amino group on the support. The functional amino support material was then treated with glutaraldehyde and then the enzyme(s) to produce coupling. The procedure for glutaraldehyde coupling was a modification of the immobilization procedure suggested by the Corning Company (Anon., 1973) and was as follows. To 1g of silanized support material, enough 2.5% aqueous glutaraldehyde solution was added to cover the support. This was placed in a vacuum desiccator evacuated by aspiration to remove air and gas bubbles from the pores. It was left under vacuum at room temperature for 30–60 min. The reaction was then allowed to continue at atmospheric pressure for another 30–60 min after which the glutaraldehyde solution was decanted and the support material thoroughly washed with deionized distilled water. Immobilization of enzyme(s) was then carried out by adding 1 ml of buffer solution (citrate-phosphate buffer, pH 7.0) containing the enzyme(s) to the support. The final concentrations of citric acid and dibasic sodium phosphate were 6.5 mM and 87 mM respectively. The enzyme concentrations varied as described below. The mixture was put on ice in a desiccator and a vacuum drawn. After 30–60 min of vacuum, the pressure was released, and the reaction was allowed to proceed on ice for an additional 30–60 min. The support was then washed with 20 ml portions of distilled de-ionized water and finally with this volume of the buffer to be used for storage or assay. Washing was carried out until no enzymic activity could be detected in the wash. Usually this was five times with each.

Enzyme assays

Both glucose oxidase and catalase were assayed in an oxygen polarograph (Gilson Medical Electronics model KM Oxygraph). All assays unless otherwise stated were done at pH 5.5, using citrate (22 mM)-phosphate (57 mM) buffer prepared according to the methods of Gomori (1955). The polarographic method of assay was used for both enzymes because of the ease of the technique and its high sensitivity. The methods of enzyme assay(s) were as follows.

Catalase

The activity of singly immobilized and soluble catalase was determined by assaying with hydrogen peroxide (5×10^{-4} M) and is expressed as picomoles oxygen/mg-sec. The buffer was flushed with N_2 gas before use.

Glucose oxidase

With singly immobilized or soluble glucose oxidase, the glucose oxidase activity was determined by assaying the sample with glucose (13.9 mM) in the presence of air-saturated buffer (0.25 mM O_2). It was also possible to assay singly immobilized or soluble glucose oxidase with glucose and excess soluble catalase in the presence of oxygen. The initial rate of oxygen depletion is one-half of what it would be if excess catalase had not been present since the catalase would regenerate one-half mole of O_2 from each mole of H_2O_2. Excess catalase is defined as that amount of enzyme such that addition of more enzyme has no effect on the rate of O_2 utilization by glucose oxidase. We generally used catalase at 100 times the activity of the glucose oxidase. Use of excess catalase allows the determination of glucose oxidase without purification in preparations contaminated with catalase, as is usually the case with commercial samples of glucose oxidase.

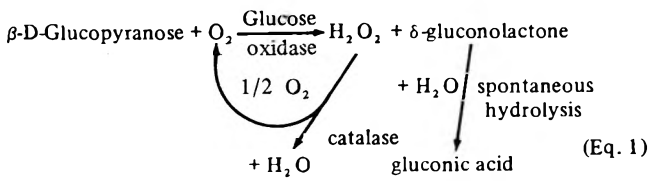
With the immobilized two-enzyme system, the general procedure was to first assay the overall reaction using air-saturated buffer containing glucose. Then catalase activity was assayed by the addition of hydrogen peroxide as described above. Finally, the assay for glucose oxidase was carried out in air-saturated buffer in the presence of glucose and excess soluble catalase. Between each assay, the test medium was removed by aspiration, and the catalyst particles washed several times with buffer solution. By sequentially assaying the immobilized enzymes with first glucose and oxygen, followed by hydrogen peroxide, and then glucose and oxygen in the presence of excess catalase, it was possible to evaluate the rate of the overall reaction and the catalase and glucose oxidase activities individually. Care must be taken in that most commercial glucose oxidase preparations contain some catalase. Glucose oxidase may be purified by a column chromatographic technique (Weibel and Bright, 1971).

After assaying with excess soluble catalase, it was necessary to remove all residual catalase activity left in the reaction cell before subsequent assays were undertaken. Repeated rinsing with distilled deionized water and/or buffer was not sufficient to eliminate adsorbed catalase. Treatment with a diluted, commercial bleach solution (1:4 v/v; pH 12.4) in the reaction compartment for 1–3 min followed by several rinsings with distilled de-ionized water and buffer eliminated adsorbed catalase.

The same basic procedure was used when the two enzymes were immobilized to the same support material or when the enzymes were individually immobilized to separate particles (mixed catalyst). In the case of the soluble system, however, the washing procedure could obviously not be used. For every assay, both the catalase and glucose oxidase activities had to be individually adjusted. The standard pH of all assays was 5.5 unless otherwise indicated.

RESULTS

THE SEQUENTIAL REACTION catalyzed by the glucose oxidase-catalase system is shown in Equation 1:



(Eq. 1)

Beta-D-glucose in the presence of oxygen is converted to the delta-gluconolactone which hydrolyzes spontaneously to gluconic acid. The hydrogen peroxide produced is converted to oxygen and water by catalase. The reaction is not only sequential but also cyclic since 1/2 of the molecules of oxygen consumed by the glucose oxidase reaction are regenerated during the splitting of hydrogen peroxide by catalase. For most of our work in the studies of efficiences, one pH was chosen, 5.5. This is the optimal pH of glucose oxidase and the beef liver catalase also shows good activity (approximately 80% of maximal) at this pH. The pH is also a reasonable one for potential utilization in food systems.

We define efficiency of the system as how well the second enzyme (catalase) in the reaction sequence utilizes the intermediate produced by the first enzyme in the sequence. By comparing the oxygen consumption during the glucose oxidase reaction to the oxygen consumption in both the glucose oxidase-initiated sequential reaction in the presence of catalase and also in the presence of excess catalase, it is possible to determine how much of the potentially available hydrogen peroxide generated by the glucose oxidase is being utilized by the catalase. This is illustrated in Figure 1. The lower curve gives the oxygen consumption that would be seen if glucose oxidase were the only enzyme immobilized to the support. This is a calculated value obtained by determining the glucose oxidase activity in the presence of excess catalase as illustrated by the uppermost curve. The first order rate constant is then determined and doubled to determine the actual glucose oxidase activity. This procedure is followed since most all commercial preparations of glucose oxidase contain some contaminating catalase which would lead to an error in the determination of glucose oxidase. By adding excess catalase to all samples, errors caused by variable amounts of contaminating catalase can be eliminated. The central curve represents the overall oxygen consumption of the glucose oxidase-catalase immobilized system. If catalase is completely inactive (0% efficient) then the rate of oxygen consumption would be equal to that of the lower curve. If the immobilized catalase were capable of utilizing all of the hydrogen peroxide produced by the glucose oxidase (100% efficient) the rate of oxygen consumption by the dual enzyme catalyst would be equal to that in the presence of added excess soluble catalase. The greater the difference between the glucose oxidase consumption of oxygen and that in the sequential reaction, the greater the catalase efficiency. This "catalase efficiency" may then be calculated by taking the difference in initial rates between the glucose oxidase activity (bottom curve) minus that of the glucose oxidase-initiated overall sequential reaction and dividing this by the difference between the glucose oxidase consumption curve and that in the presence of excess catalase, i.e., the bottom and top curves. The ratio of the differences X 100 is what is defined as "catalase efficiency." It is defined in terms of initial reaction rates and assumes the absence of any intermediate (H_2O_2) at the beginning of the reaction.

Effect of variation of enzymic activities

Nickel-impregnated silica alumina catalyst was prepared containing different glucose oxidase and catalase activities by varying the amounts of enzymes placed into the immobilizing solution (Bouin et al., 1975). Glucose oxidase and catalase activity as well as the overall activity of the system was analyzed and the percent efficiency (percent of maximal rate of hydrogen peroxide utilization) was determined (Table 1). Three comparisons were made. Samples A, B and C were prepared using a constant amount of catalase with decreasing concentrations of glucose oxidase in the immobilizing solution. Samples A, D, and E contained a constant ratio of glucose oxidase to catalase in the immobilization media but decreasing absolute amounts by factors of 10. Samples A, F and G were prepared using decreasing amounts of catalase and holding the amount of glucose oxidase in the immobilizing solution constant.

When the glucose oxidase concentration in the immobilizing solution was kept constant, glucose oxidase activity on the support material was also essentially constant. When the ratios of the enzymes in the immobilizing solution were kept constant, the ratio of activities on the support material also were relatively constant. However, when the glucose oxidase concentration was decreased and the catalase concentration maintained at a constant level during immobilization, the catalase activity was seen to increase on the support. The explanation for this is probably that because of the high turnover number of catalase, many fewer molecules are required to give

Table 1—Catalase efficiencies[a] of the dual system of glucose oxidase and catalase immobilized to nickel-impregnated silica alumina

Sample	Conc of enzyme in immobilizing solution, M		Initial enzyme activity[b]		Activity ratio Cat/GO_x	Catalase efficiency (%)
	Cat	GO_x	Cat	GO_x		
A	3.4×10^{-7}	5.3×10^{-6}	182.5	81.0	2.3	78.5
B	3.4×10^{-7}	5.3×10^{-7}	230.0	50.7	4.5	85.0
C	3.4×10^{-7}	5.3×10^{-8}	264.0	9.1	29.0	93.6
D	3.4×10^{-8}	5.3×10^{-7}	108.5	59.4	1.8	60.7
E	3.4×10^{-9}	5.3×10^{-8}	22.5	10.1	2.2	14.9
F	3.4×10^{-8}	5.3×10^{-6}	72.2	83.0	0.9	54.5
G	3.4×10^{-9}	5.3×10^{-6}	18.6	82.4	0.2	34.3

[a] Catalase efficiency is defined as the % H_2O_2 utilization compared to a system containing excess soluble catalase.
[b] The activities of both enzymes are expressed in picomoles of O_2/mg·sec.

the same amount of activity as glucose oxidase. Thus, in most of these immobilizations, the glucose oxidation concentration was much higher than the catalase concentration. When the glucose oxidase concentration was decreased by a factor of 10- and then 100-fold, there was less competition for the binding sites on the support for the few catalase molecules and significantly more of them bound. The same effect was not seen when the amount of catalase was decreased in the immobilization solution because in all cases, the total number of molecules of catalase was relatively small compared with the number of glucose oxidase molecules. Therefore, there was little competition from the catalase for glucose oxidase binding.

As the glucose oxidase activity on the support (samples A, B and C) decreased (and thus the ratio of glucose oxidase to catalase activity decreased), the percent catalase efficiency increased. Since the percent efficiency was high at all levels, it is difficult from these data to determine whether the increase in percent efficiency is due to the increase in catalase activity on these supports or to the decrease in the glucose oxidase activity, thus providing less of the hydrogen peroxide intermediate for the catalase enzyme.

When the glucose oxidase activity was maintained at a constant level, (samples A, F and G) and the catalase activity decreased (thus the ratio of glucose oxidase to catalase increased) the percent catalase efficiency went down. This is not unexpected and means simply that with the lower activity of the second enzyme of the reaction sequence, the conversion of the intermediate to the final produce was less efficiently carried out.

The efficiency of the system decreased when the absolute activities of the two enzymes decreased, although the ratio of the glucose oxidase to catalase activity was essentially constant (samples A, D and E). This decrease in efficiency was highly significant. Our explanation for it is that when the enzymes are present at the higher activities, the intermediate hydrogen peroxide is more likely to be acted on before escaping from the pores of the particulate matrix than in the case of particles with lower activities where the hydrogen peroxide has a greater chance to excape from the microenvironment of the second enzyme into the bulk phase of the solution. Once in the bulk phase, it is likely that there will be a concentration gradient of hydrogen peroxide decreasing from the particles into the bulk phase so that hydrogen peroxide would be unlikely to diffuse back to the particles. Thus, less of the hydrogen peroxide is converted to oxygen and the system works less efficiently.

Effect of pH on efficiency

The effect of immobilization on the pH-activity spectrum of glucose oxidase is shown in Figure 2 and that of catalase in Figure 3. There is essentially no effect of immobilization on the activity response of glucose oxidase to pH. The pH optimum of catalase remains constant on immobilization at about 7, but activity of immobilized catalase does not decrease as

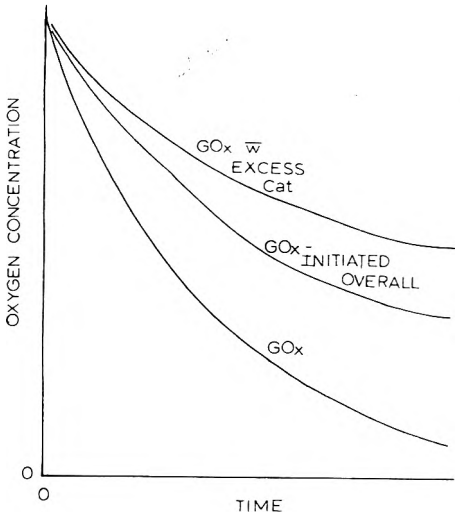


Fig. 1—O_2 consumption with time by glucose oxidase in the absence (GO_x) and presence of excess (GO_x w excess Cat) and less than excess (GO_x-initiated overall) catalase.

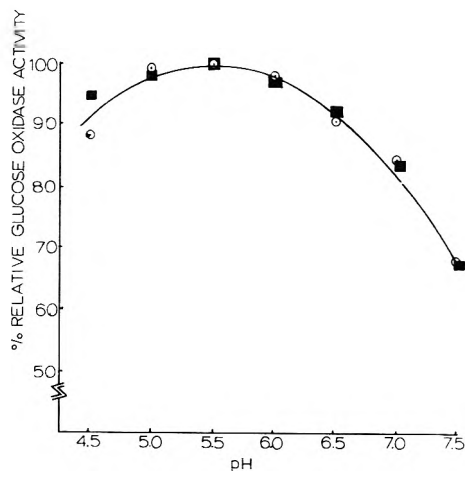


Fig. 2—Effect of pH on activity of glucose oxidase. ○—Soluble enzyme; ■—immobilized enzyme.

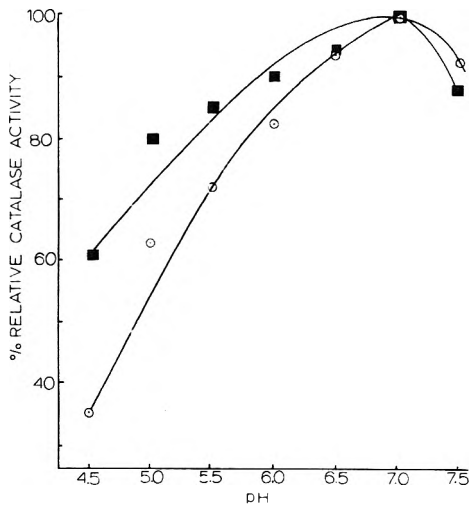
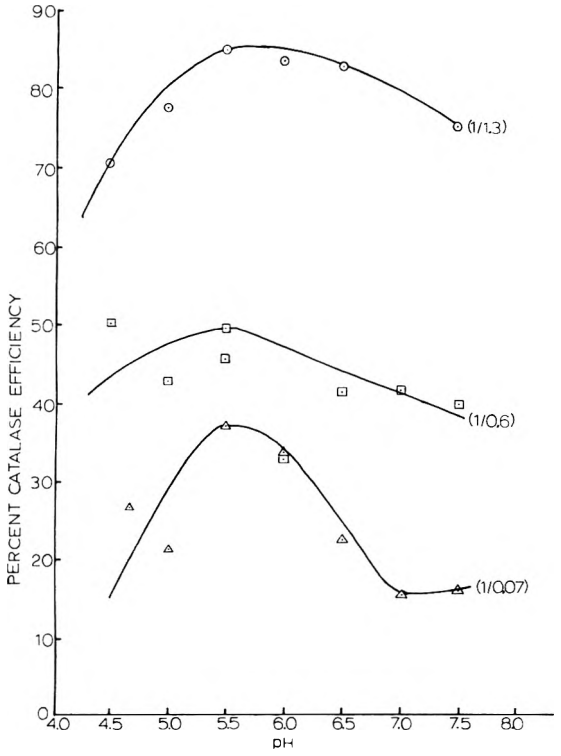


Fig. 3—Effect of pH on activity of catalase. ○—Soluble enzyme; ■—immobilized enzyme.



	Activity, picomoles/mg-sec		Ratio
	GO_x	Cat	GO_x/Cat
○	43	56	1/1.3
□	61	36	1/0.6
△	78	5.4	1/0.07

Fig. 4—Effect of pH on percent catalase efficiency of dual immobilized catalyst with varying glucose oxidase and catalase activities.

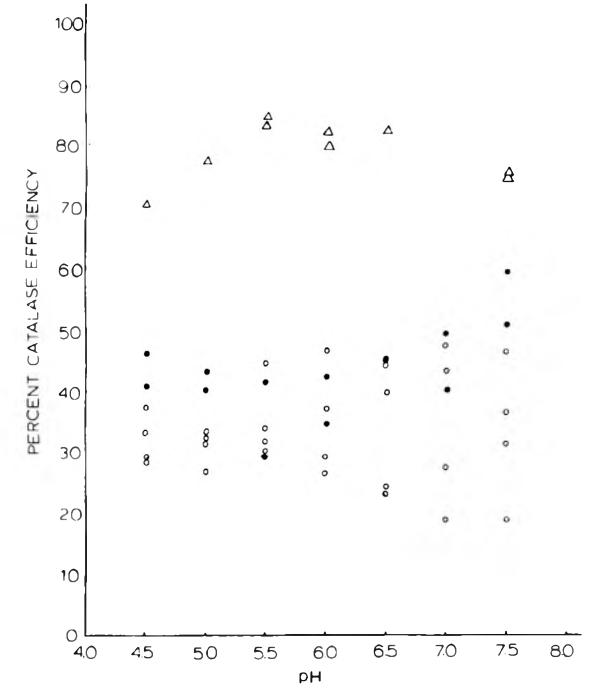


Fig. 5—Effect of pH on percent catalase efficiency of dual (△), mixed (●), and soluble (○) glucose oxidase-catalase systems. The total activities of glucose oxidase and catalase were maintained constant at 43 and 56 units, respectively, in 1.8 ml for all three samples.

fast with decreasing pH as does the activity of soluble catalase.

The efficiency of our dual enzyme preparation was determined as a function of pH at three different ratios of glucose oxidase to catalase activities (Fig. 4). The ratios were 1:1.3, 1:0.6, 1:0.07. The exact activities used are given in the legend to Figure 4. The activities were all determined at pH 5.5. Thus, the ratio indicated was true only at pH 5.5. The ionic strength in the solution (approx 0.1) was sufficiently high to minimize ionic interactions between substrate and particle surface.

As would have been expected from the data described above, the lower the ratio of glucose oxidase to catalase, the greater was the percent efficiency at all values of pH. It appeared from the upper and lower curves that there was a peak of the efficiency at approximately 5.5. We have also drawn the middle curve showing a peak at that pH; however, the data could be fitted to a straight line just as easily. The important point to make, however, is that for all three cases, there was no increase in efficiency as the pH was increased above 5.5

In another series of experiments, the relationship of percent efficiency to pH was determined for three systems. These data are shown (Fig. 5) for the two enzymes immobilized to the same support (dual), each enzyme immobilized to a separate support (mixed) and a soluble (sol) system. In all cases, the activity of each preparation was adjusted to the same total number of glucose oxidase and catalase units. The ratio used was approximately 1 to 1.3 (glucose oxidase to catalase), and the activities approximately matched those indicated in Figure 4, that is, approximately 43 units of glucose oxidase and 56 units of catalase in 1.8 ml. The activities of the enzymes in all the systems were adjusted to these values at pH 5.5. Thus, the activities and the ratios used apply only exactly at this pH. The results in Figure 5 point to two conclusions. The first of these is that at equal enzymes activities at all pH values the dual immobilized system is more efficient than either the mixed or the soluble systems. The other point is that neither the soluble nor the mixed system show an optimal efficiency at pH 5.5 as does the dual immobilized system.

DISCUSSION

ALTHOUGH the majority of the work with immobilized enzymes has dealt with organic carriers, more interest is being directed towards the use of inorganic support materials. This is due to their better flow properties in reactors, their stability to microorganisms and environmental conditions such as pH and ionic strength, and their good dimensional stability. Much of the work on immobilization to inorganic supports has involved various types of porous glass, which is fairly expensive. We chose to examine a relatively inexpensive support of the type used in the petroleum cracking industry. We chose this particular support as one out of many for several reasons. One, it has very large pore sizes from 2–40 microns. Of the several we had examined it was a relatively poor binder of enzyme. These two considerations together with the small particle size which we used, would tend to minimize diffusion restrictions in the system and allow kinetic factors to predominate.

The external diffusional resistance in the immobilized enzyme catalyst was evaluated by the procedure of O'Neil (1972). He related the maximal rate of mass transfer to a particle (Eq. 2) to the chemical reaction rate of the enzyme-catalyzed reaction assuming Michaelis-Menten rate law (Eq. 3).

$$R_{Max} = 2\pi D d_p S \qquad \text{(Eq. 2)}$$

where R_{Max} ≡ maximal mass transfer rate (mole/sec-particle);
D ≡ molecular diffusion coefficient for substrate (cm^2/sec)
d_p ≡ sphere diameter (cm) having same surface area as particle;
S ≡ substrate concentration in bulk phase (moles/cm^3).

$$r = (8.7 \times 10^{-6}\ \rho\ d_p^3)\frac{V_{Max}S}{K_M + S} \qquad \text{(Eq. 3)}$$

where r ≡ chemical reaction rate (mole/sec-particle);
V_{Max} ≡ maximal reaction velocity (mole/sec-particle);
K_M ≡ Michaelis constant (moles/ cm^3);
ρ ≡ density of the catalyst (g/cm^3).

By comparing the maximal mass transfer rate to the chemical reaction rate, one can calculate the diameter of particle which will be small enough such that external film diffusion limitations will not be important. Substituting the values for our support and the corresponding activities and particle characteristics into these equations, we concluded that there should be no external diffusional resistance for our particles if they have smaller diameters than 300 microns. The calculations assumed no movement of particles relevant to the solution. Since there is bound to be some, the calculated particle diameter will represent a minimum value. Since we were working at particle sizes smaller than 300 microns, external film diffusion can be eliminated as a factor.

Thus, the effects which we observed on the increase of efficiency with increasing absolute activities of the enzymes at a constant ratio (Table 1) must be ascribed to internal pore diffusional effects. Also, the superiority of the dual enzyme system over the mixed and soluble systems which were observed at all pH values can be ascribed to an internal diffusion effect. In the case of the dual enzyme system, the intermediate (hydrogen peroxide) produced by the glucose oxidase can be acted on by catalase before it has time to diffuse from the interior of the support particle. This system allows the buildup of the concentration of H_2O_2 in the vicinity of the catalase thus increasing the apparent activity of the catalase and leading to a greater efficiency of the system. Diffusion restrictions work against the mixed catalyst since the intermediate has to diffuse out of one particle into the bulk phase and then into the other particle containing the second enzyme. The diffusional effects of the dual enzyme system are not operative in the homogeneous soluble enzyme system. Hence, the dual system is also superior to the soluble enzyme system.

Gestrelius et al. (1972) studied the pH-activity profile of the two-enzyme system of amyloglucosidase and glucose oxidase immobilized to Sepharose. Although immobilization of the enzymes did not change the pH optimum for either enzyme, the optimum for the sequential two-enzyme reaction was displaced towards the alkaline side. This effect was explained on the basis that the increase in the concentration of the intermediate in the microenvironment of the enzyme has the same effect as increasing the activity of the first enzyme in the reaction sequence, viz., it produces more intermediate. Increasing the activity of the first enzyme in the reaction sequence makes the overall rate of the reaction sequence more dependent on the second enzyme and thus shifts the pH dependence of the overall reaction closer to that of the second enzyme. In this case the glucose oxidase had the higher optimal pH and thus there was an alkaline shift in the pH optimum of the overall reaction sequence.
relative to the glucose oxidase activity as the pH was raised. According to the data cited in Table 1, this should lead to a greater catalase efficiency. In the case of the mixed immobilized and soluble systems, there was some indication that there

In the work reported here, we did not see a comparable phenomenon. Glucose oxidase was the initial enzyme in our reaction sequence and the catalase was the second enzyme. The activities of the enzymes were adjusted at pH 5.5. Since that is the optimal pH of glucose oxidase but not the optimal pH for catalase (pH 7.0), the catalase activity should increase might be an increase in activity with increasing pH although there was much scatter in the data. At least there was no obvious maximum at pH 5.5 as was observed with the dual system. The reason for these observations is not clear. One possible explanation is that the glucose oxidase reaction is relatively more rate-limiting in the dual system. This could come about if the diffusion of glucose in the particle was the rate-limiting factor. If this were so, then glucose oxidase could remain the rate-limiting enzyme at all pH values since the diffusion of glucose would most likely be independent of pH. The efficiency of the system would remain dependent on the glucose oxidase activity. This would not be seen in the homogeneous soluble system where diffusion of glucose presumably would be no problem. This phenomenon was not seen in the mixed system where a second factor may become rate-limiting. Although the diffusion of glucose would still be as slow as in the dual system, the diffusion of hydrogen peroxide out of the glucose oxidase particles into the bulk phase and then into the catalase particle may become important. This double diffusion could require a greater time than the single diffusion of glucose in the dual system. Thus, the glucose oxidase activity could be rate-limiting at all pH values only in the dual enzyme system.

We conclude that the dual enzyme system should be more efficient in any industrial reactor than either the mixed catalyst or analogous soluble homogeneous enzyme system. We also conclude that the functioning of the dual enzyme system will depend on the absolute activities of enzymes that are obtained in the insoluble support as well as the ratio. The effects of pH may be complex and will have to be closely examined for each individual system. The dual enzyme catalyst can also be used as a model for some biological systems where more than one enzyme is attached to a surface or imbedded in a membrane.

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Ms received 8/28/75; revised 11/14/75; accepted 11/20/75.

Paper No. 1088, Massachusetts Agricultural Experiment Station, University of Massachusetts, Amherst. This research supported in part from Experiment Station Project No. 197 and National Science Foundation Grant No. GI 34976, RANN program.

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REACTIONS BETWEEN AMINO ACIDS AND ORGANIC ACIDS: REACTION OF TRYPTOPHAN AND PYRUVIC ACID

ABSTRACT

A reaction between pyruvic acid and tryptophan, which produced yellow products, was found in this investigation. The isolation and identification of the compounds produced by this interaction were carried out by paper chromatography, UV, IR and NMR spectroscopy, and organic synthetic procedures. Four compounds were isolated: three were identified as 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid, an isomer of 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid and harman. The compound which was not fully identified, was a yellow polymer. The decrease in concentration of pyruvic acid and tryptophan due to this interaction was analyzed by an automatic organic acid analyzer and an automatic amino acid analyzer. It was found that the ratio of pyruvic acid to tryptophan used in this reaction was approximately 1.6:1. This reaction was found to proceed via a 1:1 molecular reaction between tryptophan and pyruvic acid to form 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid and its yellow isomer followed by the formation of a yellow polymer produced by the interaction of the yellow isomer of 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid and pyruvic acid.

INTRODUCTION

NONENZYMATIC BROWNING has been the subject of numerous investigations, but these reactions are incompletely understood. The pigments formed are high molecular weight polymers whose identification is difficult to determine. Therefore, the browning reactions appear to be complicated not only due to the nature of the final products, but also to the mechanisms of the numerous reactions. Several hundred papers have discussed this phenomenon; however, colored compounds which cause the browning have so far not been identified. Browning reactions between amino acids and sugars, due to caramelization of polyhydroxycarbonyl compounds, or to oxidative degradation have been reported (Hodge, 1953; Reynolds, 1965). Browning, caused by the interaction of organic acids and sugars, has been described (Lewis et al., 1949; Livingston, 1953). However, the browning due to the interaction between organic acids (not sugar acids) and amino acids has not been reported on to date. The worked described in this paper involves an investigation of the discoloration caused by the interaction between pyruvic acid and tryptophan, two common food constituents.

The interaction between pyruvic acid and tryptophan has been reported by Tschesche et al. (1959). They were, however, interested in synthesis and identification at that time. This work is concerned with the formation of colored compounds which may affect food quality. As well, a decrease in the concentrations of both pyruvic acid and tryptophan may effect the acidity and nutritional value of a food.

The decrease in concentration of pyruvic acid and tryptophan due to their interaction was quantitatively analyzed in this investigation. Isolation and identification of interaction products was also carried out. Four compounds were isolated and three of them were identified. They were 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid, a yellow isomer of 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid and harman. One compound which remained unidentified was a yellow polymer.

MATERIALS & METHODS

Preparation and separation

The interaction solution was prepared by mixing tryptophan (50 μmole/ml) with pyruvic acid (150 μmole/ml) in 20 ml of distilled water in a glass container and storing at room temperature overnight. A white crystalline compound was formed in a yellow solution. The crystalline compound was collected on filter paper, washed with distilled water and dried in a desiccator for confirmative identification and for further synthesis.

The filtered yellow solution was developed on 3 MM filter paper (46 × 37 cm) with benzene-methanol-acetic acid-water (15:65:5:20) at 20°C. Four bands were isolated and each band was eluted with methanol and evaporated to dryness under vacuum. Each sample was then further developed with 5% NH_4OH. After development the individual bands were eluted with methanol and evaporated to dryness under vacuum for identification.

Identification of isolated compounds

Paper chromatography. The R_f value of each compound isolated from the interaction between pyruvic acid and tryptophan was determined by comparison with authentic (Harman, K&K Laboratories Inc.) and synthesized compounds in the following solvents systems:

BMAW: Benzene-methanol-acetic acid-water (15:65:5:20)
5% NH_4OH: 30% concentrated ammonia was diluted with water by volume.
5% HOAc: Glacial acetic acid was diluted with water by volume.
3% NH_4Cl: 3g of ammonium chloride were dissolved in 100 ml water.

Ultraviolet absorption spectra. Spectral measurements were made on a Perkin-Elmer 450 Spectrophotometer using a 1-cm cuvette. The samples were dissolved in methanol for measurement.

Infrared absorption. Absorption spectra were made on the Perkin-Elmer 337 spectrophotometer using a KBr solid, mixed with the sample.

Nuclear magnetic resonance. Absorption spectra were measured by dissolving the 30-mg sample with CD_3OD-$CDCl_3$.

Melting point. The melting point was determined by the capillary tube method.

Organic synthesis. (1) Synthesis of 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid. 1.0g of tryptophan was added to 80 ml distilled water and heated to complete dissolution. 3 ml of pyruvic acid were added after the solution cooled to room temperature. White crystalline needles were formed in the solution after standing overnight. The solution was filtered and the crystals remaining on the paper were washed with distilled water. In this manner 0.42g of white crystalline needles were obtained (Tschesche et al., 1959).

(2) Stereoisomerization of 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid. 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid obtained from E.1 was heated at 100°C for 30 min. Yellow crystals were obtained.

(3) Synthesis of 1,2,3,4-tetrahydroharman-3-dicarboxylic acid. 0.3g of tryptophan was dissolved in hot distilled water and cooled, then treated with 0.1 ml acetaldehyde and 0.1 ml acetic acid. After standing overnight, colorless needles were formed. The solution was filtered and the residue on the filter paper was washed with distilled water in order to isolate the crystals.

(4) Synthesis of harman. 0.28g 1,2,3,4-tetrahydroharman-3-carboxylic acid was dissolved in 72 ml of boiling water. 14 ml of 10% potassium dichromate solution was added. When a cloud appeared, 1.4 ml acetic acid was added. After heating for 1 min the solution was

cooled to room temperature. This solution was treated with 14 ml of 5% sodium sulfite to reduce the excess reagent. After making the mixture alkaline with sodium carbonate, it was extracted with ether. Crystals were obtained upon concentration of the extract (Jacobs and Craiz, 1936). The oxidation of 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid to harman was achieved by following the same procedure.

(5) Synthesis of 3,4-dihydroharman-3-carboxylic acid. From tryptophan and glycolaldehyde: 1g of tryptophan was dissolved in 40 ml hot water. 0.33g glycoaldehyde and 0.5 ml of acetic acid were added to the solution which was cooled to room temperature. After standing overnight, crystalline needles of 1-hydroxymethyl-1,2,3,4-tetrahydroharman-3-carboxylic acid were obtained. 0.18g of this acid was treated with 1 ml of 95% H_2SO_4 in 2.5 ml water. After being stored at room temperature for 2 days the solution was placed in a steam bath for 2 hr. An additional 0.5 ml of 95% H_2SO_4 was added and the solution was allowed to stand for 2 hr at room temperature. It was then diluted and neutralized with NaOH. The neutralized solution was extracted with ether, concentrated and further purified by paper chromatography (5% NH_4OH). This procedure was a modification of the method of Spensor (1959).

From 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid: 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid (0.4g), synthesized previously, was dissolved in 100 ml hot water, treated with 0.2g of palladium-charcoal and 4 drops of concentrated HCl, and refluxed for 2 hr. The solution was filtered, concentrated and basified with 2N NaOH. The filtrate was extracted with ether, the extract acidified with 2N HCl, concentrated to near dryness and filtered. The filtrate was treated with 5 ml of 1% $AuCl_3$ and allowed to stand 1 hr, then filtered. The residue was dissolved in methanol and treated with 15% HCl and 1 ml of $AuCl_3$ solution. Tetrachloroaurate of harmalan-3-carboxylic acid was precipitated. The free acid was liberated by eliminating the gold from tetrachloroaurate of harmalan-3-carboxylic acid by bubbling H_2S through the flask. The free acid solution was filtered and evaporated to obtain 3,4-dihydroharman-3-carboxylic acid (Tschesche and Jenssen, 1960).

Addition of pyruvic acid, acetic acid, citric acid and tryptophan to 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid. In order to detect any other reactions of 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid with other compounds 20 mg were dissolved in 10 ml hot water and allowed to cool at room temperature. 60 mg each of pyruvic acid, acetic acid, citric acid and tryptophan were added separately to this solution in four beakers. Each solution was allowed to stand at room temperature under vacuum for 1 day until dry. Each dried sample was dissolved with methanol and developed on 1 MM filter paper (46 × 37 cm) with 5% NH_4OH (5 hr), 3% NH_4Cl (10 hr), 5% HOAC (4 hr), and BMAW (12 hr) and compared with the yellow isomer of 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid. The formation of a yellow-colored compound other than this isomer might indicate that interaction or polymerization occurred.

Quantitative analysis of pyruvic acid and tryptophan from the interaction of pyruvic acid and tryptophan

A solution was prepared by mixing pyruvic acid (15 μmole/ml) with tryptophan (2.5 μmole/ml). Pyruvic acid analysis was conducted weekly during storage using an Automatic Organic Acid Analyzer (Waters Associates, Milford, Mass.). The theory and operating principles of this apparatus are based on the work of Kesner and Muntwyler (1966).

The detailed operation, preparation of silica gel, column packing, preparation of indicator and calibration were described completely by Lin et al. (1970). Two chambers were used in this study containing pure chloroform and 30% (v/v) tert-amyl-alcohol/chloroform, respectively. Tryptophan was also analyzed weekly with a Beckman Model 120 C Amino Acid Analyzer (Spinco Div. of Beckman Instruments, Inc. Palo Alto Calif.). The theory of operation, preparation of the buffer solutions and ninhydrin reagent, packing of the column, and other procedures are based on the *Handbook for the Beckman Model 120 C Amino Acid Analyzer.* Calculation of the peak area was based on the H × W method (Beckman Handbook). The calculation of the concentration of tryptophan was based on the following equation.

$$\mu\text{mole/ml of tryptophan} = \frac{\text{peak area of sample} \times 2.5}{\text{peak area of standard}}$$

RESULTS & DISCUSSION

FOUR COMPOUNDS were isolated from the interaction between pyruvic acid and tryptophan by paper chromatography. Three of them were identified as follows:

Compound 1

This compound was identified as 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid (Fig. 1) by the following evidence. (a) The melting point was 225–227°C, the same as reported by Tschesche et al. (1959). (b) By oxidizing with potassium dichromate, harman was produced. Harman was also produced by oxidizing 1,2,3,4-tetrahydroharman-3-carboxylic acid. The formation of harman may be seen in Figure 2. (c) NMR showed the singlet of the methyl group for this compound whereas 1,2,3,4-tetrahydroharman-3-carboxylic acid (K&K Laboratories Inc.) showed the doublet (Fig. 3). Therefore, it was concluded that the compound under investigation had a carboxy group attached at position 1. (d) IR Spectra (Fig. 4) showed that this compound had a secondary amine group (3400 cm^{-1} and 750 cm^{-1}), a carboxy group (3000–3300 cm^{-1}) and a carbonyl group (1600–1750 cm^{-1}). (e) UV Spectra (Fig. 5) showed absorption maxima at 272 nm, 280 nm and 290 nm. These results were similar to those of Tschesche et al. (1959).

Compound 2

This compound was identified as a yellow isomer of 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid by the following evidence. (a) Melting point: 226–228°C. (b) Harman was produced by oxidizing with potassium dichromate. (c) NMR spectra showed the singlet of the methyl group described pre-

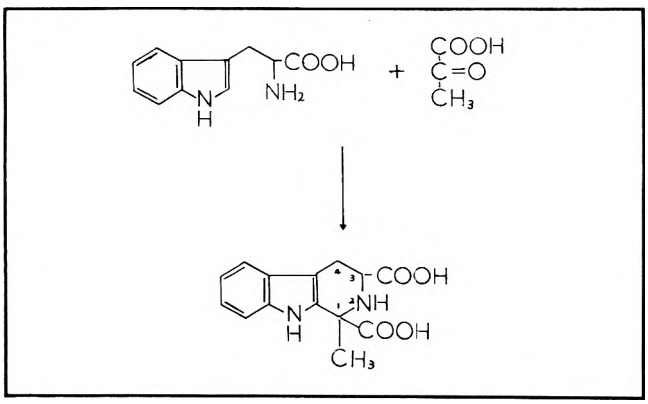

Fig. 1–Formation of 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid from interaction between pyruvic acid and tryptophan.

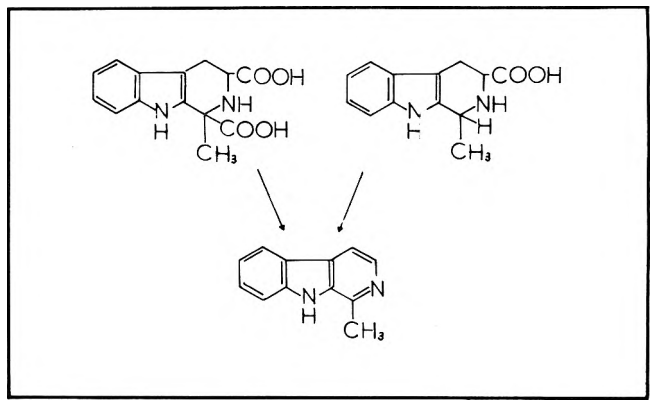

Fig. 2–Formation of harman from the oxidation of 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid and 1,2,3,4-tetrahydroharman-3-carboxylic acid.

viously for compound 1. (d) IR spectra showed the same absorption as compound 1 (Fig. 4). (e) UV spectra showed an absorption maxima at 350 nm in addition to that shown previously at 272 nm, 280 nm and 290 nm (Fig. 5). The absorption at 350 nm might be due to a change in structural conformation.

Therefore, it was concluded that compound 2 is the isomer of compound 1. However, compound 2 is a yellow solid and stable, whereas compound 1 is white and unstable.

Compound 3

This compound was identified as harman by comparison with authentic harman (K&K Laboratories Inc.) by paper chromatography in four different solvent systems (Table 1).

Compound 4

This compound was yellow, sticky and difficult to purify. Therefore, identification was not established in this investigation. Two compounds are presumed to make-up this compound. One of them is 3,4-dihydroharman-3-carboxylic acid. The other is a polymerized compound.

3,4-dihydroharman-3-carboxylic acid was synthesized from 1,2,3,4-tetrahydroharman-1,3-carboxylic acid and from tryptophan and glycolaldehyde. However, compound 4 was not identified to be 3,4-dihydroharman-3-carboxylic acid by paper chromatography.

The solution prepared from 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid (THDA) and pyruvic acid formed compound 4 wherease other solutions prepared from acetic acid, citric acid and tryptophan with THDA did not produce this compound. As a matter of fact compound 4 was formed by polymerizing THDA with pyruvic acid. Unfortunately, identification was not accomplished.

The R_f values of isolated, oxidized and synthesized compounds may be seen in Tables 1, 2, and 3, respectively. Compound 1 was unstable and gradually isomerized to compound 2 (yellow) by storing at room temperature or by heating. When 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid (compound 1) was converted to a yellow isomer, it became more soluble in water and more stable. When the interaction solution between pyruvic acid and tryptophan was processed at 116°C for 19.5 min and stored, a white crystalline compound (compound 1) was not obtained. Therefore, it was concluded that the colored compounds in this solution were due to the yellow isomer of 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid and the yellow isomer.

Table 4 shows that during the reaction with tryptophan pyruvic acid decreased rapidly during the early stage of storage and then reached steady state. About 4.00 μmole/ml of pyruvic acid were utilized during storage. The decrease in concentration of tryptophan may be seen in Table 5. The amount of tryptophan decreased rapidly initially, and then gradually during the rest of the storage. The tryptophan completely disap-

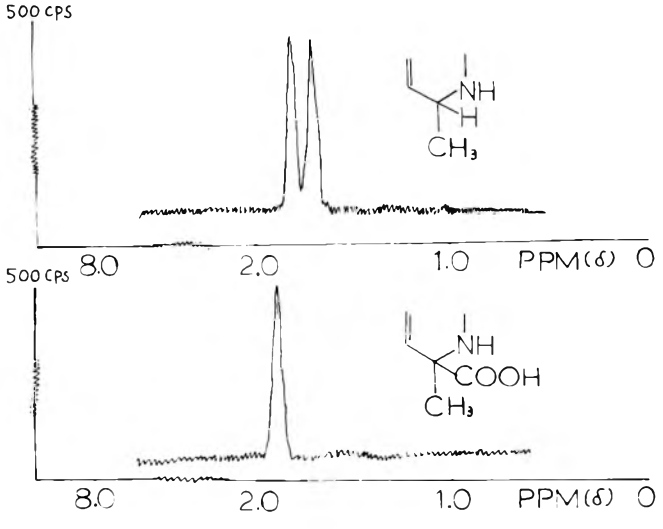

Fig. 3—NMR spectra of compound 1 (lower) and 1,2,3,4-tetrahydroharman-3-carboxylic acid (upper). Compound 1 was isolated from interation solution containing pyruvic acid and tryptophan.

Table 1—R_f values of the isolated compounds obtained from the interaction of pyruvic acid and tryptophan

Isolated compounds	R_f values (X 100) 5% NH_4OH	3% NH_4Cl	5% HOAC	BMAW	Authentic compounds
1 (white)	83	64	67	63	1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid
2 (yellow)	54	51	64	72	Isomer of 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid
3 (white)	10	41	69	82	harman
4 (yellow)	76	73	78		

Table 2—R_f values of oxidized compounds obtained by the oxidation of compounds isolated from the reaction between pyruvic acid and tryptophan

Oxidized compounds	5% NH_4CH	3% NH_4Cl	5% HOAC	BMAW	Authentic compounds
5[a]	10	41	69	82	harman
6[b]	10	41	69	82	harman
7[c]	10	41	69	82	harman
8[d]	50	43	59	70	3,4-dihydroharman-3-carboxylic acid
9[e]	52	45	59	70	3,4-dihydroharman-3-carboxylic acid

[a] Oxidized from compound 1
[b] Oxidized from compound 2
[c] Oxidized from 1,2,3,4-tetrahydroharman-3-carboxylic acid
[d] Synthesized from 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid
[e] Synthesized from 1-hydroxy-1,2,3,4-tetrahydroharman-3-carboxylic acid which is product from interaction between tryptophan and glycoaldehyde

Table 3—R_f values of compounds formed from the interaction between 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid and other compound

Mixture solutions	5% NH_4OH	3% NH_4Cl	5% HOAC	BMAW	Compounds identified
THDA[a]	57	50	65	74	
THDA + tryptophan	57	56	65	74	THDA
THDA + acetic acid	57	56	65	74	THDA
THDA + citric acid	57	56	65	74	THDA
THDA + pyruvic acid	57	56	65	74	THDA
	76	73	78	74	compound 4

[a] THDA: yellow isomer of 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid

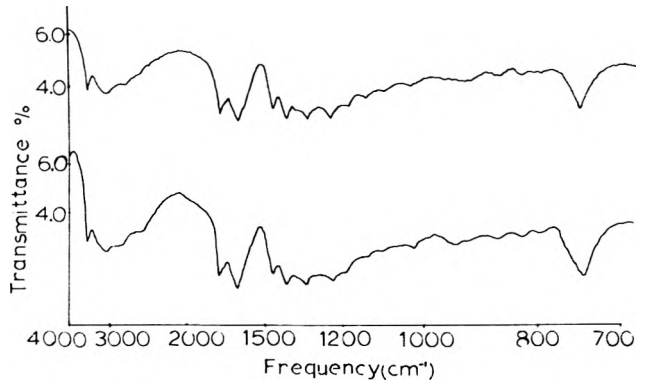

Fig. 4—IR spectra of compound 1 (upper) and compound 2 (lower) isolated from the interaction solution containing pyruvic acid and tryptophan.

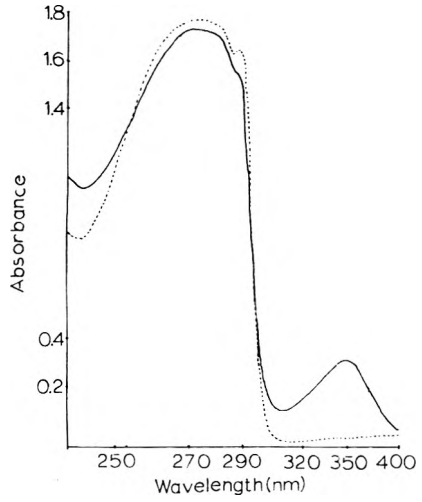

Fig. 5—UV spectra of compound 1 (dotted line) and compound 2 (solid line) isolated from interaction solution containing pyruvic acid and tryptophan.

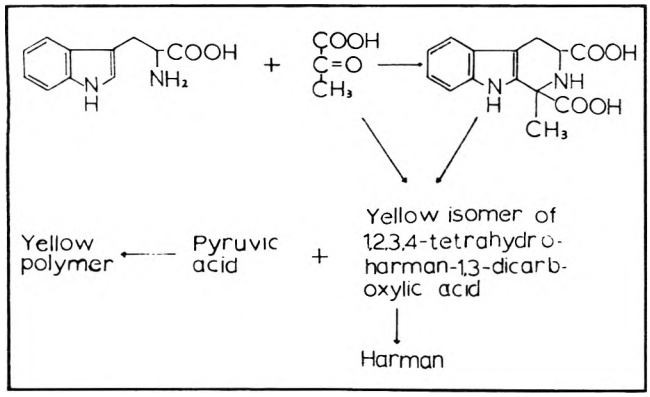

Fig. 6—Formation of colored compounds due to the interaction between tryptophan and pyruvic acid.

Table 4—Concentration of pyruvic acid in solution with tryptophan during storage at 24° C

Storage (wk)	Conc decrease (μmole/ml)
0	1.04
2	1.36
3	2.26
6	4.06
7	3.74
8	3.74
9	4.08
10	4.12

Table 5—Concentration of tryptophan in solution with pyruvic acid during storage at 24° C

Storage (wk)	Conc decrease (μmole/ml)
0	2.40
1	1.65
2	1.25
3	0.50
4	0.40
6	0.11
7	0.06
8	0

peared in 8 wk (Table 5). Therefore, about 1.50 μmole/ml (4.00 μmole/ml–2.50 μmole/ml) more pyruvic acid was consumed in this interaction. According to the rapid decrease of the concentration of both pyruvic acid and tryptophan during the early stages of storage, it would seem that most of the major interaction compounds were formed at that time. Initially, intermediate compounds were produced by a 1:1 molecular reaction between pyruvic acid and tryptophan as shown by the amount of each used during this period. Then polymerization took place due to the interaction of an intermediate compound with pyruvic acid during the latter part of storage. At the same time natural oxidation may have occurred during the long storage due to the very small amount of harman formed at this stage. When the interaction solution containing pyruvic acid and tryptophan was analyzed by paper chromatography during different stages of storage, only 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid and its isomer were identified after 2 wk storage. Other compounds were produced during longer storage. It was concluded, therefore, that polymerized compounds were formed gradually during the latter period of storage.

The reaction postulated for the formation of the yellow compounds may be seen in Figure 6. The isomer of 1,2,3,4-tetrahydroharman-1,3-dicarboxylic acid and polymerized compounds showed a yellow color, whereas other compounds were devoid of color.

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Paper No. 2011, Massachusetts Agricultural Experiment Station, University of Massachusetts at Amherst. This research supported in part from Experiment Station Project No. 192 and the Glass Container Manufacturers Institute, New York, N.Y.

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REACTIONS BETWEEN AMINO ACIDS AND ORGANIC ACIDS: REACTION OF TRYPTOPHAN AND ALPHA-KETOGLUTARIC ACID

ABSTRACT

This study involved an investigation of the discoloration found to be caused by the interaction between α-ketoglutaric acid and tryptophan. Many products were formed by this interaction, but only the five compounds in the highest concentration were isolated: three were blue, one green and one a brown-yellow. Unfortunately, due to instability, these compounds were not identified. However, the concentration of both α-ketoglutaric acid and tryptophan, utilized during the reaction, were analyzed. The results indicated that the ratio of α-ketoglutaric acid to tryptophan utilized was approximately 3:1. From these results along with various identification procedures (paper chromatography, UV-Vis, and IR spectroscopy, and organic synthesis) a pathway is postulated for the formation of the colored compounds in this interaction.

INTRODUCTION

IN 1874 ADAMKIEWICZ described a color phenomenon which developed due to the action of concentrated sulfuric acid on egg-albumin in acetic acid solution. He found, moreover that the color obtained depended upon the concentration of the protein in the mixture. It was Adamkiewicz's view that the colored products arose from the interaction of substances liberated from the protein by the sulfuric acid and that acetic acid only modified the reaction (Fearson, 1920; Harvey et al., 1941). Rosenhein (1906) reported that formaldehyde gives rise to a characteristic color reaction with proteids in the presence of sulfuric acid containing oxidizing agents. This reaction is due to the formation of a proteid-formaldehyde compound and its subsequent oxidation. This reaction also depends upon the presence of the tryptophan group in the proteid-molecule. Fearson (1920) reported that the color (blue and red) condensation reaction due to the interaction between tryptophan and aldehyde was formed by adding hydrogen peroxide and passing hydrochloric acid gas into the mixture.

It is well known that tryptophan either alone or in peptide combination is readily oxidized by periodate (Clamp and Hough, 1965; Sklarz, 1967). The reaction of sodium periodate produced ammonia, N-formylkynurenine and a high-melting insoluble dark brown solid (Nicolet and Shinn, 1939). The reaction of tryptophan with sodium periodate in the presence of hydrochloric acid with aqueous periodic acid followed a different course, the main product being a high-melting insoluble brown solid accompanied by the formation of ammonia and dioxindolylalanine (Rivett and Wilshire, 1971). This might indicate that tryptophan can be destroyed by acid and oxidizing agents. The destruction of tryptophan during acid hydrolysis of proteins has been reported but the mechanism of destruction has not been established and end products have not been identified (Albro and Fishbein, 1971).

The formation of color from the interaction between α-ketoglutaric acid and tryptophan has not been reported to date. The aim of this investigation was to measure the decrease of α-ketoglutaric acid and tryptophan quantitatively during interaction, and to isolate and identify the colored compounds formed.

MATERIALS & METHODS

Preparation of interaction solution

The interaction solution was prepared by mixing tryptophan (50 μmoles/ml) with α-ketoglutaric acid (100 μmoles/ml) in 20 ml distilled water in a stoppered glass bottle and storing at room temperature (24°C) for 2 wk to allow color development.

Isolation of interaction compounds

When the formation of the dark precipitant stopped, the water soluble brown-colored solution was filtered and washed with distilled water. The dark-colored compounds remaining on the filter paper were then solubilized with methanol. Both the water soluble and insoluble fractions were evaporated to dryness under vacuum at room temperature for further isolation. Following evaporation both fractions were developed and separated on 3 MM filter paper (46 × 57 cm) with benzene-methanol-acetic acid-water (15:65:5:20) for 12 hr. Four bands were isolated from the water insoluble fraction and one band from the water soluble fraction. Each band was eluted with methanol and evaporated to dryness under vacuum at room temperature. Each evaporated sample was further developed with 5% NH_4OH for 5 hr. After development the individual bands were eluted with methanol and evaporated to dryness under vacuum for identification.

Identification of isolated compounds

Paper chromatography. The R_f value of each isolated compound was determined on 1 MM filter paper by comparison with synthetic compounds in benzene-methanol-acetic acid-water (BMAW) (15:65:5:20) (12 hr), 5% NH_4OH (5 hr), 5% HOAc (4 hr), and 3% NH_4Cl (10 hr).

Ultraviolet and visual absorption spectra. Spectral measurements were made on a Perkin-Elmer 450 Spectrophotometer using 1-cm cuvettes. The samples were dissolved in methanol for measurement.

Infrared absorption. Absorption spectra were measured with a Perkin-Elmer 337 Spectrophotometer using a KBr solid mixed with the sample. The confirmation of the functional group aids in the identification.

Organic synthetic procedures. (a) Oxidation of isolated compounds: The initial interaction solution, water soluble fraction and isolated bands from the water insoluble fraction were oxidized with potassium dichromate. About 0.28 mg of sample was dissolved in 72 ml of boiling water. 14 ml of 10% potassium dichromate solution was added. 1.4 ml acetic acid was added. After boiling 1 min the solution was cooled to room temperature. The solution was then treated with 14 ml of 5% sodium sulfite to reduce the excess reagent. After making the mixture alkaline with sodium carbonate, it was extracted with ether and evaporated to dryness under vacuum at room temperature (Jacobs and Craiz, 1936). The dry sample was dissolved in methanol. The compounds in the methanol solution were separated and isolated by paper chromatography on 3 MM filter paper for 5 hr using 5% NH_4OH. The isolated compounds were compared with another oxidized compound which was prepared by the reaction of tryptamine and α-ketoglutaric acid followed by the same oxidation procedure described previously.

(b) Synthesis of norharman from the interaction between glyoxylic acid and tryptophan: 0.25g tryptophan was dissolved in 20 ml of hot water. After the solution was cooled to room temperature, 0.75g of glyoxylic acid was added and the solution allowed to stand overnight. The white crystals that formed were filtered and oxidized by the procedure described in the previous paragraph. The oxidized compound was identified by comparison with standard norharman (Aldrich Chem. Co. Inc.) via paper chromatography.

Reaction of α-ketoglutaric acid with acetyltryptophan, N-methyltryptophan and tryptamine. 0.06g tryptophan was mixed with 0.02g of acetryptophan, N-methyltryptophan and tryptamine, respectively, in 4 ml distilled water. Each solution was stored at 24°C for 1 month for the development of color.

Quantitative analysis of α-ketoglutaric acid and tryptophan after interaction

The interaction solution was prepared by mixing tryptophan and α-ketoglutaric acid at a concentration of 2.5 μmoles/ml of 10μmoles/ml respectively. This solution was analyzed weekly to determine the concentrations of α-ketoglutaric acid and tryptophan during storage.

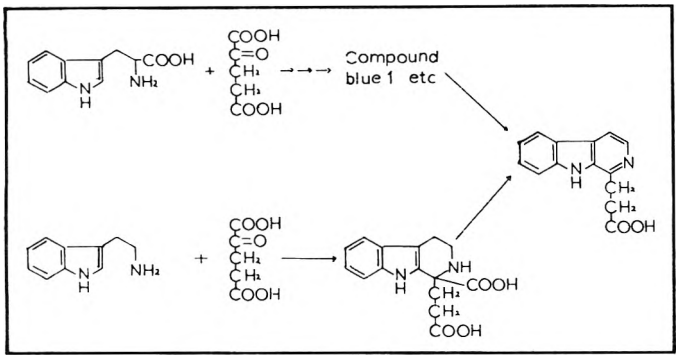

Fig. 1—Formation of 1-carboxyethylnorharman from isolated blue 1 etc. and from 1-carboxyethyl-1,2,3,4-tetrahydronorharman-1-carboxylic acid by oxidation with potassium dichromate.

Table 1—R_f values of the isolated compounds from the interaction between α-ketoglutaric acid and tryptophan

Isolated compounds	R_f values (X 100) 5% NH_4OH	3% NH_4Cl	5% HOAc	BMAW
Blue 1	1			8
Blue 2	5			22
Blue 3	15			45
Green	55			70
Brown-yellow	77	65	69	70

Table 2—R_f values of oxidized compounds from isolated compounds from interaction between α-ketoglutaric acid and tryptophan

Oxidized compounds	5% NH_4OH[a]	3% NH_4Cl[a]	5% HOAc[a]	BMAW[a]	Tentative[a] compound
X[b]	9	35	53	80	1-carboxyethyl-norharman
Y[c]	9	35	53	80	1-carboxyethyl-norharman
TA X K[d]	9	36	54	80	1-carboxyethyl-norharman
Water soluble fraction	9	35	53	80	1-carboxyethyl-norharman

[a] Detected by UV light
[b] X — Oxidized from blue 1
[c] Y — Oxidized from blue 2
[d] TA X K — Oxidized from interaction between tryptamine and α-ketoglutaric acid

Analysis of α-ketoglutaric acid. This acid was analyzed by an automatic organic acid analyzer (Waters and Associates, Inc., Milford, Mass.). The principles on which this analyzer is based are described by Kesner and Muntwyler (1966). The detailed operations, preparation of the silica gel, column packing, preparation of indicator, composition of solvent gradient and calibration were described completely by Lin et al. (1970).

Two chambers of chloroform and 30% (v/v) tert-amyl-alcohol/chloroform respectively were used in this study.

Analysis of tryptophan. Tryptophan was analyzed by a Beckman Model 120 C Amino Acid Analyzer (Spino Division of Beckman Instruments, Inc., Palo Alto, Calif.). The theory of operation, preparation of the buffer solutions and ninhydrin reagent, packaging of the column, and other procedures are discussed in detail in the handbook for the Beckman Model 120 C Amino Acid Analyzer.

Calculation of the peak area was based on the H × W method (*Handbook for the Beckman Model 120 C Amino Acid Analyzer*). The calculation of the concentration of tryptophan from the peak of a sample was based on the following equation:

$$\mu\text{mole/ml of tryptophan in sample} = \frac{\text{Peak area of sample} \times 2.5}{\text{Peak area of standard}}$$

RESULTS & DISCUSSION

A NUMBER of compounds were formed from the interaction between α-ketoglutaric acid and tryptophan. Only five of these have been isolated. Four of them were from the water insoluble fraction and one from the water soluble fraction. However, none of these was identified. The color and R_f values in various solvents of the isolated compounds are shown in Table 1. There were three blue compounds, one green and one brown-yellow. Since their instability caused changes during storage, and purification was difficult, identification was not established.

Blue 1 and blue 2 were the only compounds from Table 1 which seemed to be stable and were in sufficient concentration to investigate further. Blue 1, blue 2 and the original water extracted fraction were oxidized with potassium dichromate and compared to the postulated compound, 1-carboxyethylnorharman (CEH) produced by the interaction of tryptamine and α-ketoglutaric acid followed by oxidation of the intermediate, postulated to be 1-carboxyethyl-1,2,3,4-tetrahydronorharman-1-carboxylic acid (CTHC) (Fig. 1).

The R_f values of each of the products of oxidation are shown in Table 2. From these results it seems apparent that the same compound is being formed by all the reactions. This seems to indicate that the pathway of formation is similar to that shown in Figure 1. Moreover, it may be postulated that blue 1, blue 2 and the water soluble fraction contain an intermediate similar to CTHC which would be 1-carboxyethyl-1,2,3,4-tetrahydronorharman-1,3-dicarboxylic acid.

IR Spectra (Fig. 2) indicate that blue 1 and blue 2 possess a carboxy group (3000–3500 cm^{-1}), a methylene group (2850–3000 cm^{-1}), and (1420–1485 cm^{-1}) and a carbonyl group (1600–1750 cm^{-1}).

UV-Visible Spectra showed some differences between blue 1 and blue 2. Blue 1 showed maximum absorption at 337 nm and 350 nm in UV, and 610 nm and 650 nm in Vis. whereas blue 2 only showed maximum absorption at 610 nm and 660 nm in Vis. This might indicate that both blue 1 and blue 2 are conjugated structures. However, UV-Vis. spectra of both blue 1 and blue 2 does not provide positive information for identification.

Table 3 shows changes in the concentration of α-ketoglutaric acid from the interaction between α-ketoglutaric acid and tryptophan. The concentration of acid decreased gradually during storage and reached a steady state. The consumption of acid in the steady state was about 1.60 μmole/ml. Table 4 shows the decrease in the concentration of tryptophan from

the interaction between α-ketoglutaric acid and tryptophan during storage. It was found that tryptophan did not decrease in concentration continuously during storage but only in the initial stages of the reaction. Table 4 indicates that only about 0.50 μmole/ml of tryptophan were consumed in the solution with 10 μmoles/ml of α-ketoglutaric acid and 2.5 μmoles/ml of tryptophan. Therefore, the utilization of α-ketoglutaric acid in the interaction solution during storage is about three times that of tryptophan.

These results suggest that the formation of the color was caused by an initial reaction of α-ketoglutaric acid and tryptophan forming an intermediate which then reacted with α-ketoglutaric acid to form polymers. Based on these results a possible pathway for the formation of color is shown in Figure 3.

In pathway D, α-ketoglutaric acid forms a pyrrole ring by reacting with tryptophan (primary amine) according to the Paal-Knorr synthesis (Badger, 1961). However, the formation of the pyrrole ring requires heat. Therefore, this mechanism cannot apply to the formation of colored compounds which occur without heating, as in part of this investigation.

Pathway C depicts the formation of an intermediate compound involving two molecules of tryptophan and one molecule of α-ketoglutaric acid similar to a mechanism proposed by Hopkins-Cole (Friedman and Finely, 1971) whereby 1 mole of glyoxylic acid and 2 mole of tryptophan interact. But norharman was formed by oxidation, with potassium dichromate, of the product(s) of the interaction between tryptophan and glyoxylic acid (Table 5). Therefore, the Hopkins-Cole type reaction does not indicate the entire array of possible compounds. In addition, color formation has not been observed by the interaction between α-ketoglutaric acid and N-methyltryptophan during storage. It is apparent that the primary amino group is blocked by the methyl group and reaction is inhibited. On the other hand, color has been formed by interactions between α-ketoglutaric acid and acetyltryptophan, and between α-ketoglutaric acid and tryptamine where the primary amine is not blocked. This indicates that the reaction is dependent upon the reactivity of the primary amine group which would not be involved if pathway C was correct. Therefore, pathway C is impossible in this case.

The formation of an intermediate compound via pathway B is acceptable. This intermediate is similar to the Maillard type, formed by the interaction between fructose and an amino acid (Reynolds, 1965). However, the rearrangement and subsequent polymerization is not feasible since the formation of an enol-form, an ene-form and a furfural-form from α-ketoglutaric acid is impossible because it contains no hydroxy

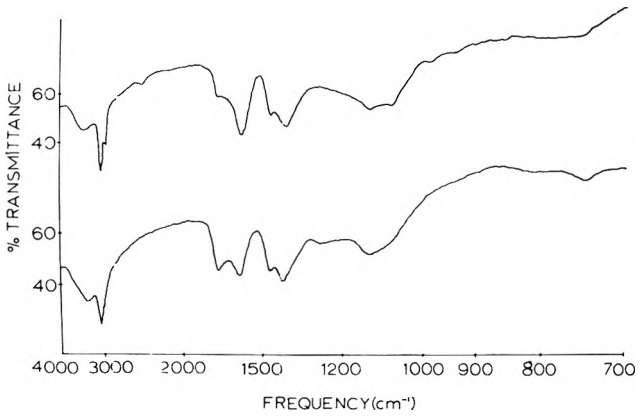

Fig. 2–IR spectra from the compound blue 1 (lower) and compound blue 2 (upper) isolated from interaction between α-ketoglutaric and tryptophan.

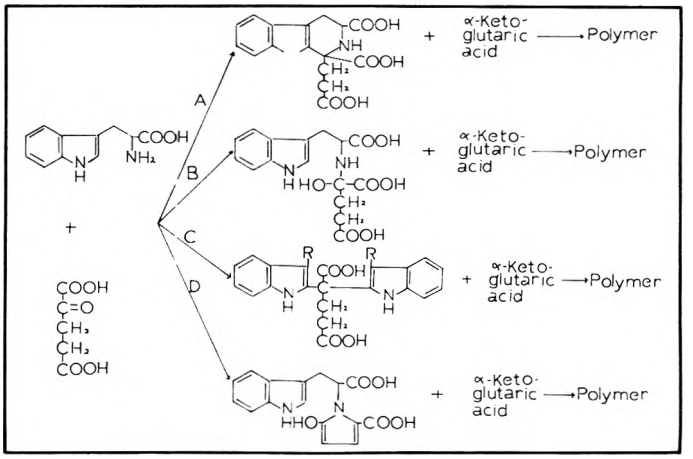

Fig. 3–Postulated pathways of the formation of colored compounds via interaction between α-ketoglutaric acid and tryptophan during storage. Under the experimental conditions of this investigation it would appear that D and C are impossible for the reaction under study.

Table 3–Concentrations of α-ketoglutaric acid from the interaction between α-ketoglutaric acid (10 μmole/ml) and tryptophan (2.5 μmole/ml) during storage at 24°C

Storage (wk)	Conc decrease (μmole/ml)
0	0.30
2	1.10
3	1.30
6	1.60
7	1.40
8	1.52
9	1.56
10	1.52

Table 4–Concentration of tryptophan from interaction between α-ketoglutaric acid (10 μmole/ml) and tryptophan (2.5 μmole/ml) during storage at 24°C

Storage (wk)	Concentration (μmole/ml)
0	2.08
1	2.25
2	2.37
3	2.10
4	2.25
6	2.17
8	2.05
10	2.05

Table 5–R_f values of oxidized and interacted compounds between glyoxylic acid and tryptophan

Isolated compounds	R_f values (X 100)[a]			
	5% NH_4OH	3% NH_4Cl	5% HOAc	BMAW
1,2,3,4-tetrahydronorharman-1,3-dicarboxylic acid	79	65	67	61
Oxidation of above compound	13	37	53	76
Authentic norharman	13	37	55	77

[a] Detected uncer UV light

groups. On the other hand, fructose has hydroxy groups on all carbons except the keto carbon. Hodge (1953) reported that if the carbon 2 hydroxy of aldose was substituted, then the derivative remained white and stable after storage for 2 hr at 25°C. Thus, it was shown that blocking the Amadori rearrangement of an N-substituted glycosylamine blocks the browning which would otherwise occur. Wolfrom and Tipson (1959) reported that the presence of an alpha-hydroxy ketone is essential if the rearrangement for browning is to proceed. The rearrangement in the side chain of the intermediate compound from interaction between α-ketoglutaric acid and tryptophan is unlikely. Unfortunately, the crystalline intermediate compound was not isolated in this investigation and further studies of the possible pathways have not been carried out.

Pathway A is the other possible route whereby colored compounds may be formed. However, the possible polymerization of this intermediate compound is uncertain.

The formation of colored compounds from the interaction between α-ketoglutaric acid and tyyptophan might follow both pathway A and B, or follow A alone. In the former case pathway B would precede A. In order to establish precise pathways for the formation of colored compounds, the identification of intermediate compounds and colored compounds must be advanced more fully.

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Ms received 12/3/74; revised 12/13/75; accepted 12/16/75.

Paper No. 2012, Massachusetts Agricultural Experiment Station, University of Massachusetts at Amherst. This research supported in part from Experiment Station Project No. 192 and the Glass Container Manufacturers Institute, New York, N.Y.

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SENSITIVITY OF Vibrio parahaemolyticus TO SPICES AND ORGANIC ACIDS

ABSTRACT

The effects of 13 dried spices added to growth media at concentrations ranging from 0.1–1.0% were studied with respect to their bactericidal activity against *Vibrio parahaemolyticus*. Rates of growth of *V. parahaemolyticus* in culture media containing essential oils of oregano, thyme, and sassafras and in media containing citric, ascorbic, and malic acids were determined. Survival of the organism in tomato sauce adjusted to pH 4.4–6.5 and incubated at 10, 22 and 35°C were examined. Dried oregano and thyme were highly toxic to *V. parahaemolyticus* when present in growth media at a concentration of 0.5%. Essential oils of oregano, thyme, and sassafras were bactericidal at a 100 μg/ml level. Growth curves were similar in media adjusted to identical pH values, regardless of whether citric, ascorbic or malic acid was used to attain these values. No viable *V. parahaemolyticus* were detected in inoculated tomato sauce adjusted to pH 4.4 and held 24 hr at 10 and 22°C or after 4 hr at 35°C. After 24 hr, only a slight reduction in viable cell count was noted in tomato sauce adjusted to pH 5.6–6.5.

INTRODUCTION

THE ANTIMICROBIAL ACTION of onion and garlic powders and extracts has been reported by several researchers. Vaughn (1951) demonstrated fresh onion juice to be germicidal to both bacteria and yeasts while Al-Delaimy and Ali (1970) reported that filtered onion extracts were bactericidal against *Shigella dysenteriae* and *Staphylococcus aureus*. Inhibition of *Salmonella typhimurium* and *Escherichia coli* by reconstituted onion and garlic powders was reported by Johnson and Vaughn (1969). A relationship between the pungent principle of onion and its antimicrobial activity has been suggested (Wei et al., 1967). Growth of many species of zoopathogenic fungi was shown to be inhibited by aqueous extracts of garlic bulbs (Appleton and Tansey, 1975).

In addition to granulated onion, other spices such as allspice, oregano, and cassia were determined by Julseth and Deibel (1974) to interfere with the detection of *Salmonella* when pre-enrichment techniques were used. Bullerman (1974) reported that cinnamon inhibited mycelial growth and aflatoxin production by *Aspergillus parasiticus*. Out of 12 vegetable extracts examined for their suitability to support the growth of *Vibrio parahaemolyticus*, eggapple, tomato, carrot, parsley, celery and onion were shown to be inhibitory (Temmyo, 1966).

Acid pH conditions have been shown to inhibit growth of *V. parahaemolyticus*. Vanderzant and Nickelson (1972) were not able to recover the organism from inoculated shrimp homogenates adjusted to pH 1.0–4.0. A relationship between minimal pH for growth of *V. parahaemolyticus* in laboratory media and incubation temperature has been noted (Beuchat, 1973). Hydrochloric acid was used to adjust the pH of substrates examined in both of these laboratories.

Foodborne poisoning outbreaks in which *V. parahaemolyticus* has been implicated as the causative agent have been almost exclusively associated with the consumption of seafood. Seafood, especially shellfish, is often served in the form of an hors d'oeuvre or cocktail. In such cases, the product may remain at ambient temperature for several hours before being consumed. *V. parahaemolyticus* present on seafood could multiply to dangerous levels during this time, resulting in a health hazard. In light of data reported by others on the inhibitory effects of spices on microorganisms, experiments were designed to determine the effects of those spices commonly used in seafood cookery and preparation on the survival and growth of *V. parahaemolyticus*. Since lemon juice and tomato-based sauces are often used in condiments or as ingredients when preparing seafoods, the effect of reduced pH on the viability of *V. parahaemolyticus* was studied. Citric, ascorbic, and malic acids were used to adjust the pH of growth media. Survival of *V. parahaemolyticus* suspended in tomato sauce adjusted to pH 4.4–6.5 was also monitored.

EXPERIMENTAL

Test bacterium and inoculum preparation

V. parahaemolyticus strain 8700 (04:K11) was the test organism used throughout this study. The organism was originally isolated from steamed crabs which had been implicated in a food poisoning outbreak (Molenda et al., 1972).

Tryptic soy broth (Difco, Detroit, Mi.) containing 3.0% sodium chloride (TSBS), pH 7.3, served as the culturing medium for preparing *V. parahaemolyticus* inocula. All inocula consisted of 16- to 18-hr cultures of *V. parahaemolyticus* grown in TSBS at 30°C on a gyratory shaker (150 rpm).

Screening of spices for inhibitory activity

Dried spices were added individually, at various concentrations ranging to 1.0% (w/v), to TSBS containing 1.2% agar (TSAS) before sterilizing by heating at 121°C for 15 min. Spices (McCormick & Co., Inc., Baltimore, Md.) examined were Mexican chili powder (hot), powdered horseradish, fancy paprika, instant onion powder, instant garlic powder, ground cayenne (red pepper), East Indian nutmeg, Indian curry powder (mild), ground black pepper, ground mustard, ground thyme, and ground imported oregano. Gumbo filé (Zatarain's Inc., New Orleans, La.) was also tested for anti-*Vibrio* activity. *V. parahaemolyticus* (16- to 18-hr culture) was serially diluted in 0.1 M phosphate buffer (pH 7.3) containing 3.0% sodium chloride (salt buffer) and plated in TSAS (42°C) containing test spices. Plates were incubated at 35°C and generally counted after 18–24 hr. In cases where colonies developed slowly on test media, counts were made after 48–72 hr.

Inhibitory effects of spice oils

Essential oils of oregano, thyme and sassafras (Meer Corp., North Bergen, N.J.) were diluted in ethanol and added in 0.1-ml aliquots to 100 ml TSBS in 250-ml Erlenmeyer flasks to give final concentrations of 10 and 100 μg of oil per ml. Ethanol (0.1 ml) containing no essential oil was added to TSBS and served as a control. A 1-ml aliquot of an 18-hr culture of *V. parahaemolyticus* which had been diluted 10^{-3} in salt buffer was used to inoculate 100 ml of the spice oil-TSBS and control TSBS media. Cultures were incubated at 35°C in a waterbath shaker (150 rpm) (AquaTherm, New Brunswick Scientific Co., Inc., New Brunswick, N.J.). Samples were withdrawn over a 7-hr incubation period, diluted in salt buffer, and plated in TSAS. Colonies were counted after 18 hr at 35°C.

Effect of organic acids on growth of *V. parahaemolyticus*

Acids prevalent in lemons and tomatoes (citric, ascorbic, and malic) were added individually to TSBS to result in pH values ranging from 5.2–7.2 in 0.4 unit increments. Adjusted TSBS media (100 ml per 250-ml Erlenmeyer flask) were inoculated with 1 ml of an 18-hr culture of *V. parahaemolyticus* and incubated at 35° in a waterbath shaker. Samples were withdrawn over an 8-hr incubation period and absorbance at 630 nm was determined using a Spectronic 20 Spectrophotometer (Bausch & Lomb).

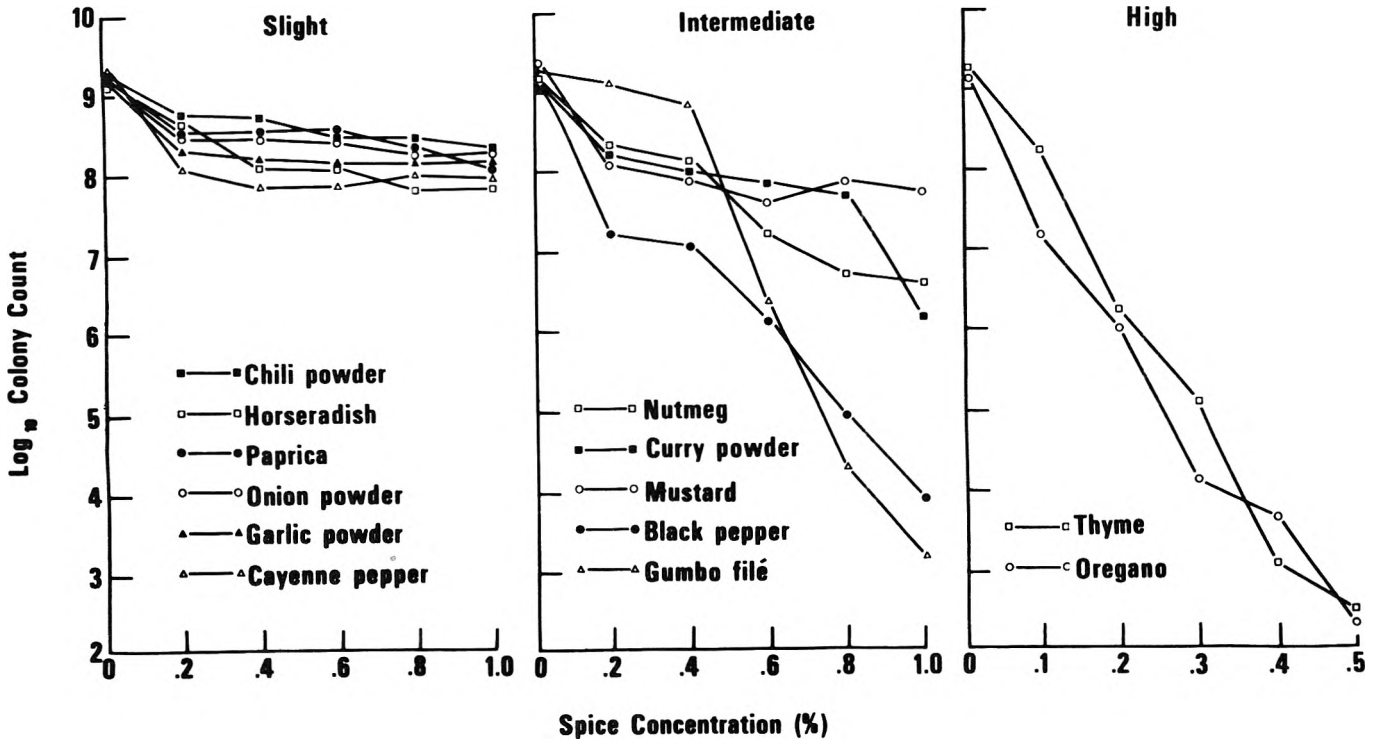

Fig. 1—Effect of spices on colony-forming ability of V. parahaemolyticus. Spices were arbitrarily grouped in three categories (slight, intermediate, and high) according to their degree of inhibition.

Survival of *V. parahaemolyticus* in tomato sauce

Sufficient sodium hydroxide was added to tomato sauce (Hunt Wesson Foods, Inc., Fullerton, Ca.) to give pH values ranging from 4.4–6.5 in 0.3 unit increments. Sauces were adjusted to 10, 22 and 35°C and inoculated (10%, v/v) with an 18-hr culture of *V. parahaemolyticus*. Slight readjustment of pH following inoculation was required in some instances. Sauces were thoroughly mixed just prior to assaying for viable cells over a period of 24 hr. Appropriate dilutions were made in salt buffer and samples were plated on TSAS. Counts were made after 18–24 hr incubation at 35°C.

Data presented in this paper represent means from three or more independent trials run in duplicate.

RESULTS

Screening of spices

The 13 spices screened for their inhibitory activities against *V. parahaemolyticus* were arbitrarily divided in three groups according to their degree of effectiveness (Fig. 1). Chili powder, horseradish, paprika, onion powder, garlic powder and cayenne exhibited slight toxicity at concentrations up to 1% in the TSAS medium. Nutmeg, curry powder, mustard powder, black pepper, and gumbo filé were moderately inhibitory over the same concentration range, while thyme and oregano were highly bactericidal when present in TSAS at a level of 0.5%.

Effect of essential oils on growth of *V. parahaemolyticus*

Viable cell counts for *V. parahaemolyticus* cultured in TSBS containing 10 and 100 μg/ml of essential oil of oregano, thyme, and sassafras are illustrated in Fig. 2. Counts from the control culture (0.1% ethanol in TSBS) were similar to those shown for cultures containing 10 μg/ml of essential oils. Thus, 10 μg/ml of the test oils did not appear to inhibit the growth of *V. parahaemolyticus*. At a concentration of 100 μg/ml, however, the three essential oils were initially bactericidal. Oregano had the greatest lethality, followed by thyme and sassafras. Growth was evident after an extended incubation period.

Effect of organic acids on growth of *V. parahaemolyticus*

Reduction in pH of TSBS through the addition of citric, ascorbic, and malic acids resulted in extended lag periods and decreased total *V. parahaemolyticus* cell production (Fig. 3). Changes in absorbance at 630 nm were not detected in inoculated TSBS adjusted to pH 5.6 and 5.2 over the 8-hr incubation period. With the exception of TSBS adjusted to pH 6.0

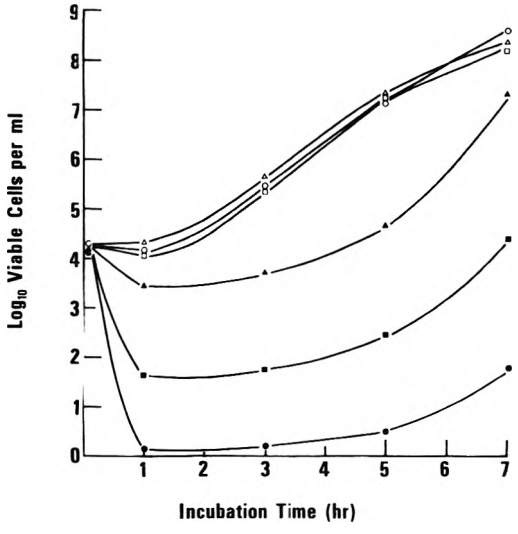

Fig. 2—Effect of essential oils of oregano (●, ○), thyme (■, □) and sassafras (▲, △) on the growth of V. parahaemolyticus in TSBS. Closed symbols indicate 100 μg/ml; open symbols indicate 10 μg/ml.

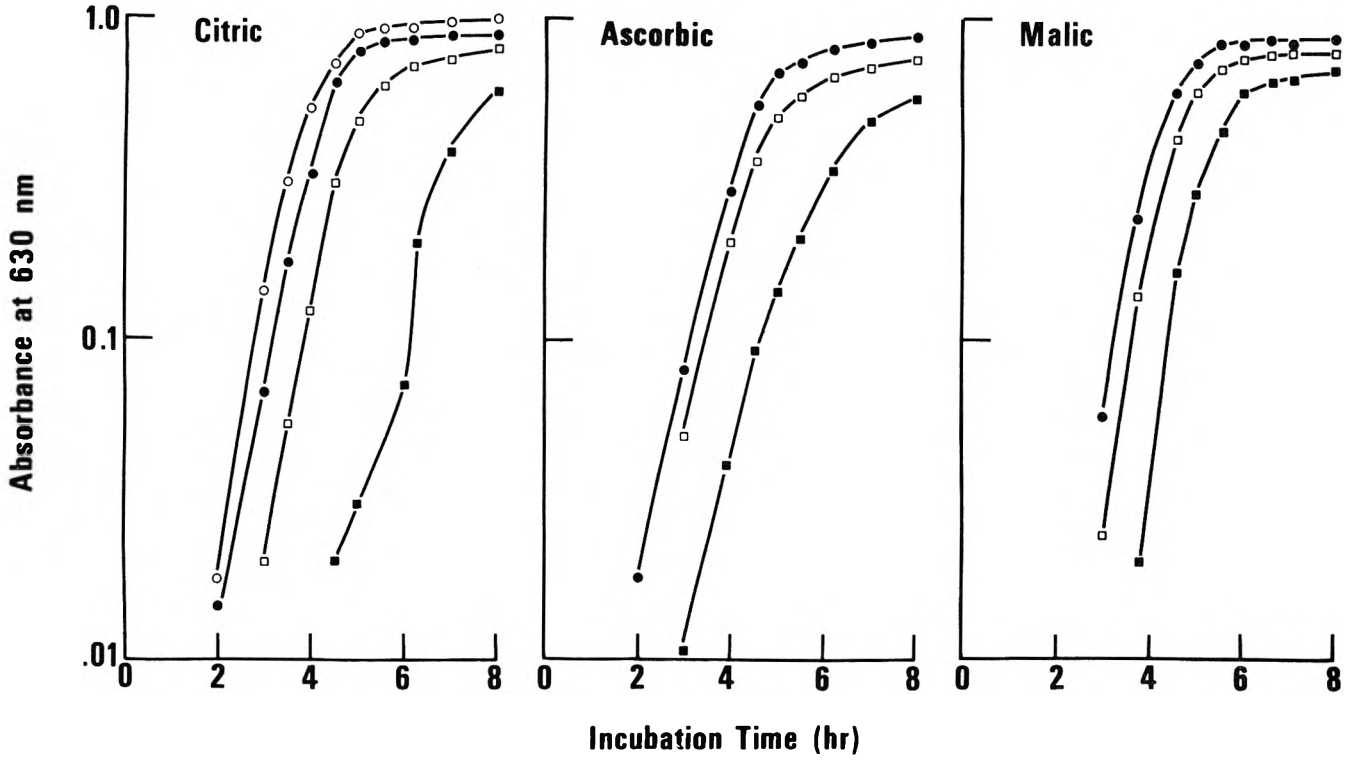

Fig. 3—Effect of organic acids on the growth of V. parahaemolyticus in TSBS. Symbols: ○, pH 7.2 (control, no acids added); ●, pH 6.8; □, pH 6.4; ■, 6.0.

with citric acid, growth curves were similar in TSBS with identical pH values, regardless of the acid used to attain these values.

Table 1 lists the amounts of citric, ascorbic, and malic acids required to reduce the pH of TSBS to levels tested in this study. Both percentages used and corresponding approximate molarities of the acids in the test media are given.

Survival of *V. parahaemolyticus* in tomato sauce

Survival curves for *V. parahaemolyticus* in tomato sauce adjusted to pH 4.4–5.3 and held at 10, 22, and 35°C for 24 hr are illustrated in Figure 4. Only slight reduction in viable cell count was noted in tomato sauce at pH 5.6–6.5 (not shown). Considering pH values of 4.4 and 4.7, increased incubation temperature resulted in greater lethality to *V. parahaemolyticus*. No viable cells were detected in tomato sauce adjusted to pH 4.4 and held for 24 hr at 10 and 22°C or after 4 hr at 35°C.

DISCUSSION

THE LOW BACTERICIDAL ACTIVITY of onion and garlic powders relative to other test spices was somewhat surprising. Acrolein is supposedly an active principle in onions and garlic (Frazier, 1967) and has been demonstrated to be germicidal to a variety of microorganisms. Temmyo (1966) reported that *V. parahaemolyticus* did not grow in onion extract containing 2% sodium chloride. However, the pH of the extract was 4.8, which in itself would be inhibitory to *V. parahaemolyticus*. Compared to other spices, dried oregano (origanum) and thyme were highly bactericidal. Gumbo filé (ground sassafras leaves) was also effective as a growth inhibitor. A characteristic common to these three spices is that they are derived from the leaves and flowering parts of plants. It is interesting to note that the remaining ten spices included in the study are derived mainly from plant seeds or roots.

The degree of anti-*Vibrio* activity of essential oils of oregano, thyme, and sassafras followed the same order as that of the dried materials. That is, oregano and thyme oils were most toxic while sassafras was least. Thymol and carvacol are the two main constituents of both oregano and thyme oils (Fenaroli's Handbook of Flavor Ingredients, 1971). These and other highly volatile phenols may be responsible for the initially high death rate of *V. parahaemolyticus* (Fig. 2). Upon volatilizing from the TSBS growth media, the bactericidal effectiveness of these phenols would be reduced. This in turn may account for the increased viable population noted to occur late in the incubation period.

The degree of inhibition of growth of *V. parahaemolyticus* in media containing citric, ascorbic, and malic acids appears to be dependent upon the pH of medium and not upon the particular acid used to achieve the pH. For example, TSBS containing 0.110% ascorbic acid supported the growth of *V. parahaemolyticus* at a rate similar to that observed in TSBS containing 0.036% malic acid (Table 1). Both media were at pH 6.8. These data are not in agreement with those reported by Mountney and O'Malley (1965). They demonstrated that the

Table 1—Percentages, with approximate molarities, of citric, ascorbic and malic acids used to adjust the pH of TSBS

	Citric acid		Ascorbic acid		Malic acid	
pH	%	M	%	M	%	M
7.2	0.012	0.0006	0.060	0.0034	0.006	0.0004
6.8	0.036	0.0019	0.110	0.0062	0.036	0.0027
6.4	0.074	0.0039	0.215	0.0122	0.065	0.0048
6.0	0.112	0.0058	0.305	0.0173	0.097	0.0072
5.6	0.143	0.0076	0.375	0.0213	0.129	0.0096
5.2	0.180	0.0094	0.447	0.0254	0.161	0.0120

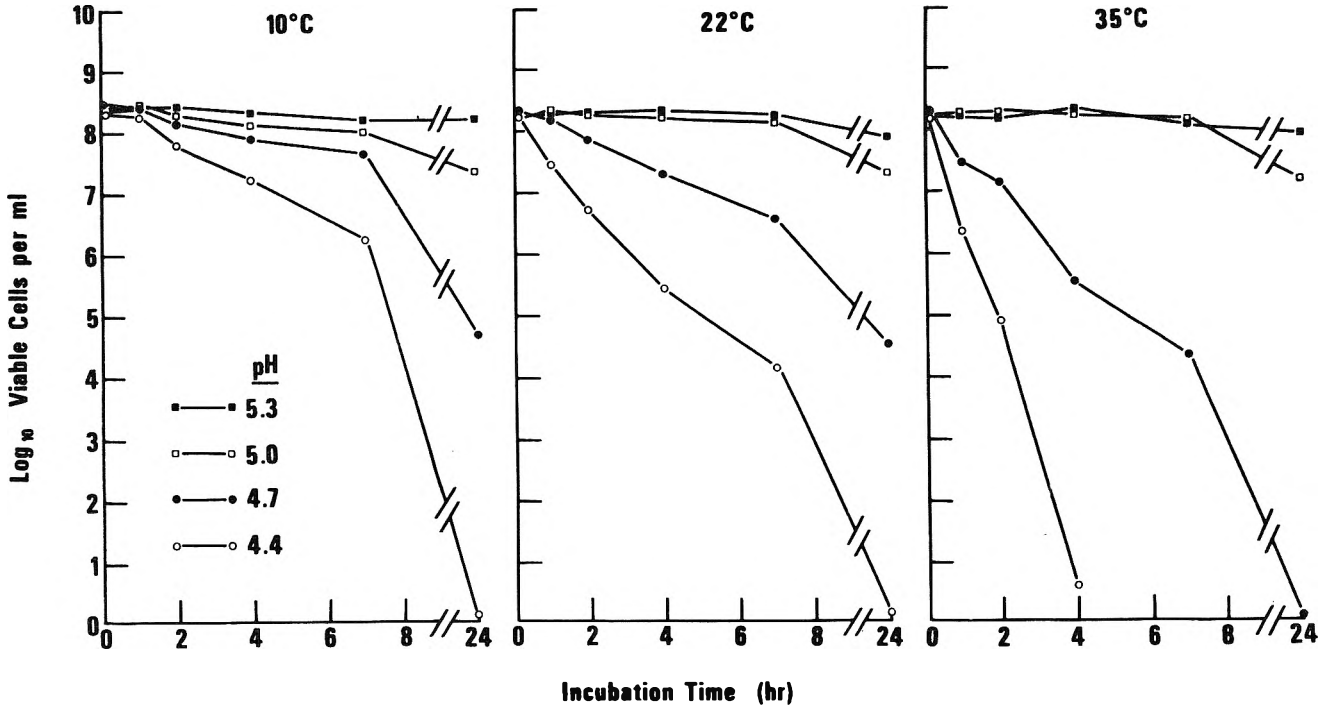

Fig. 4—Survival of V. parahaemolyticus in tomato sauce adjusted at various pH values and incubated at 10, 22 and 35° C.

degree of inhibition of growth of microflora on poultry carcasses did not depend entirely upon the pH of various immersion solutions used to treat the poultry. Total aerobic plate counts from treated refrigerated poultry meat varied, depending upon the acid used in the initial treatment. Survival and growth of specific genera of bacteria were not reported.

Bradshaw et al. (1974) reported on the viability of *V. parahaemolyticus* in a commercial seafood cocktail sauce and shrimp mixture over a 48-hr period. In sauce alone, *V. parahaemolyticus* die-off was virtually complete in only 6 hr. The rapid decline in viable cells was attributed to the acidity of the sauce (pH 3.3–3.4). Data presented here tend to agree with those of Bradshaw et al. (1974). No viable *V. parahaemolyticus* were detected in tomato sauce at pH 4.4 (the lowest pH tested) after 24 hr at 10 and 22°C or after 4 hr at 35°C. The lack of substantial reduction in viable cell counts for *V. parahaemolyticus* suspended in tomato sauce at pH 5.0–6.5 for 24 hr was unexpected. The buffering that would result from association of vibrios with shrimp tissue (Bradshaw et al., 1974) was theorized to have protected cells against the bactericidal effects of acid pH. If tomato sauce were to be combined with other ingredients before adding to seafoods contaminated with *V. parahaemolyticus*, the final pH of the prepared mixture might be 5.0 or higher. Under such conditions, the viability of *V. parahaemolyticus* would be extended. On a practical scale, however, it is unlikely that *Vibrio* would be present on seafoods at the initially high levels studied in this experiment. In any case, the important point to be made from data presented here is that *growth* of *V. parahaemolyticus* was not observed in tomato sauce adjusted to pH 4.4–6.5.

It should be noted that only one strain of *V. parahaemolyticus* was examined in this study and that considerable difference may exist in sensitivities among strains. Natural microflora other than vibrios present on seafood may also affect the survival of *V. parahaemolyticus* in the presence of added spices and organic acids. A study involving a larger number of test strains and the use of nonpasteurized seafoods should therefore be conducted as an extension of work presented here.

In summary, data indicate that organic acids such as citric, ascorbic, and malic, at levels within a range found in lemon juice and tomato products, have an inhibitory effect on the growth of *V. parahaemolyticus*. Certain spices, especially oregano, thyme, and sassafras, have a bactericidal effect on the organism when incorporated in growth media at concentrations which might be used in seafood products.

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Ms received 11/8/75; revised 1/9/76; accepted 1/12/76.

I am grateful for the technical assistance of Kelly Jones and Brenda Vaughn. Gratis samples of essential oils were supplied by Meer Corp., North Bergen, N.J.

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ISOLATION AND PURIFICATION OF Clostridium perfringens ENTEROTOXIN BY AFFINITY CHROMATOGRAPHY

ABSTRACT

Sporulating cells of certain strains of *Clostridium perfringens* produce an intracellular enterotoxin believed to be the causative agent of *C. perfringens* food poisoning. Rabbit antibody preparations to both vegetative and sporulating cell extracts were used to purify toxin by differential immunoaffinity chromatography. Solid phase immunoadsorbents were prepared by attaching antibody via α-amino groups to succinylaminoethyl Sepharose-4B to give two resins: one binding antivegetative immunoglobulin G (IgG), (V-resin), the other, antisporulating IgG, (S-resin). Sporulating cell extract was passed through resin with bound IgG to vegetative cell extract. A large protein fraction representing antigens common to both *C. perfringens* forms was retained, but toxin was not measurably absorbed to the resin as determined by erythemal activity in rabbits. The toxic fraction was then passed through resin with bound IgG to sporulating cell extract. The majority of the protein did not adhere to this resin; however, that which did, showed erythemal activity in rabbits. Disc-gel electrophoresis of the protein fraction eluted from resin with bound IgG to sporulating cell extract demonstrated the presence of five components. One elicited erythemal activity in rabbits. The maximum capacity of the V-resin column was 102 μg of bound protein/ml of resin, and of the S-resin column, 32 μg of bound protein/ml of resin. A 150-fold purification was achieved by the procedure. Resins could be used repeatedly.

INTRODUCTION

Clostridium perfringens Type A causes one of the most common types of bacterial food poisoning in humans (Hauschild, 1973). The illness is caused by an enterotoxin produced in association with sporulation and released into the environment (Duncan et al., 1972).

Clostridium perfringens food poisoning, characterized by abdominal pain and watery diarrhea, has been produced experimentally in animals (Duncan and Strong, 1969). Fluid accumulation in ligated ileal loops of animals has been correlated directly with the development of typical symptoms in monkeys and humans (Strong et al., 1971; Duncan and Strong, 1971). The enterotoxic factor causing intestinal fluid accumulation is the only toxin of *C. perfringens* known to elicit erythema in guinea pigs and rabbits when injected intradermally (Hauschild, 1970), and the erythemal reaction is accepted as a direct measure of the enterotoxin (Stark and Duncan, 1971).

Isolation of the enteropathogenic factor of *C. perfringens* has been accomplished using gel and ion-exchange chromatography (Hauschild and Hilsheimer, 1971). Purification using these techniques is a somewhat lengthy procedure, and some loss of activity (70%) is experienced. The toxic factor has also been purified with 74% recovery by ammonium sulfate precipitation and repeated gel filtration (Sakaguchi et al., 1973). An abbreviated purification technique applicable to small volumes of sample material would be desirable.

Affinity chromatography has been used to purify antigens and antibodies (Silman and Katchalski, 1966). Since vegetative cells do not produce the toxin (Duncan et al., 1972), rabbit antiserum, specific for enterotoxin, can be prepared from sporulating cell extracts (Stark and Duncan, 1971; Sakaguchi et al., 1973). Exploiting this difference, sequential absorption of sporulating cell extract antigens with immobilized forms of antivegetative IgG and antisporulating IgG was tried as a means to purify the enterotoxin. The results of this study are reported here.

MATERIALS & METHODS

Organism

Clostridium perfringens NCTC-8239, obtained from Dr. C.L. Duncan of the Food Research Institute, Univ. of Wisconsin, Madison, Wisc., was used in this study. Stock cultures of the organism were maintained on cooked meat (Difco) in RCM broth (Oxoid) at ambient (ca 25°C) temperatures.

Cell extract preparation

Vegetative cell cultures, 16-hr old, and sporulating culture held for 9 hr in sporulation broth were harvested and sonicated according to the method of Hauschild et al. (1970). The technique included extensive washing of the cells to remove culture medium, and procedures were followed closely to avoid production of antibodies to antigens in the medium. Further, independent tests showed media to be devoid of factors which would contribute significantly to erythemal activity in rabbits. These precautions eliminated the necessity of preparing antibodies to the culture medium. Spores were produced in DS medium as developed by Duncan and Strong (1967).

Morphological examination of cultures showed significant amounts of sporulating cells in 7–9 hr. Cultures were harvested after 9 hr of growth to obtain the intracellular toxin prior to the appearance of large numbers of mature spores. Sedimented cells were resuspended in 0.85% NaCl and ruptured with a Bronwill sonifier (Biosonik III, Bronwill Scientific, Rochester, N.Y.) for 40 min. The sonicated cell suspension was centrifuged at 17,300 × G for 15 min and the supernatant fluid stored frozen until used.

Antibody production

New Zealand white rabbits about 6-wk old were used to produce antiserum to crude vegetative cell extract and to crude sporulating cell extract fractionated by gel filtration on Sephadex G-100. Antiserum to cell extracts was produced by initial intramuscular injections of a 1:1 mixture of extract in 0.01M phosphate buffered saline, pH 7.2, and Freunds complete adjuvant, followed by weekly subcutaneous injections of a 1:1 mixture of the antigen in a calcium alginate adjuvant (Sodium alginate, Wilson Diagnostics, Inc., 3 Science Road, Glenwood, IL 60425). Approximately 175 μg total protein was administered in each injection. Blood samples were taken initially and after the third and fourth injections. Antibody titer was monitored using double diffusion agar plates and complete bleeding was done following the fifth week. Immunoglobulin G fractions (IgG) were precipitated from rabbit antiserum to cell extracts by adding saturated ammonium sulfate, pH 7.0, to a final concentration of 40% ammonium sulfate. The precipitate was resuspended in distilled water and again precipitated with saturated

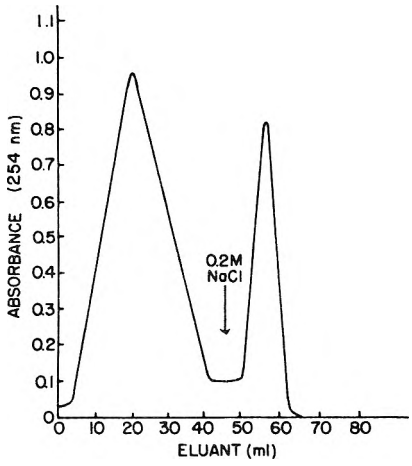

Fig. 1—Elution pattern of C. perfringens sporulating cell extract from antivegetative resin, (V-resin). Bound antigen dissociated from immobilized antibody with 0.2M NaCl.

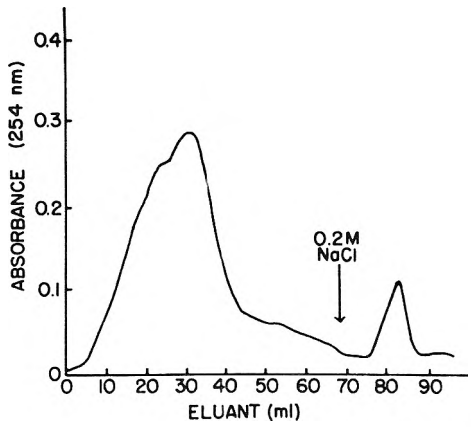

Fig. 2—Elution pattern of C. perfringens sporulating cell extract from antisporulating resin, (S-resin). Bound antigen dissociated from immobilized antibody with 0.2M NaCl.

ammonium sulfate added to a final concentration of 33%. This protein fraction was dialyzed against 0.01M phosphate buffered saline, pH 7.2, and used to prepare the specific resins for the procedure.

Immunoadsorbent resin preparation

The twice precipitated immunoglobulin G fraction (IgG) of rabbit antiserum to vegetative cell extract and to sporulating cell extract was attached via α-amino groups to succinyl aminoethyl Sepharose-4B (Cuatrecasas, 1970; Cuatrecasas and Parikh, 1972). Sepharose-4B was activated with 175 mg cyanogen bromide per ml packed gel and allowed to react with a 10-fold excess of ethylenediamine for 72 hr. Aminoethyl Sepharose-4B was then prepared exactly according to Cuatrecasas (1970), then activated by the method of Cuatrecasas and Parikh (1972). Activated gel was resuspended in buffer containing 0.1 ml IgG/ml gel and stirred 2 hr at 5°C. Immunoadsorbent resins were packed in columns 20 × 65 mm, and excess IgG was washed from the resin using 0.01M phosphate buffer, pH 7.2. Columns were stored at 5°C when not in use.

Extract elution

Sporulating cell extract was passed through the resin column which contained bound IgG to vegetative cell extract (V-resin). The material which did not adhere to the support resin was concentrated using polyethylene glycol, 20,000 to a volume of approximately 4 ml. This concentrated material was passed through a second resin column containing bound IgG to sporulating cell extract (S-resin). The eluting buffer was 0.01M phosphate, pH 7.2, containing 0.02% sodium azide. Material retained by both resins was removed using the same buffer with 0.2M sodium chloride added. Material retained by the S-resin was concentrated using polyethylene glycol 20,000.

Erythemal activity

Erythemal activity, a direct assay of the enterotoxin (Stark and Duncan, 1971), was used to evaluate the fractions from affinity chromatography. Depilated New Zealand white rabbits 6–8 wk old were given intradermal injections of 0.1 ml according to the method of Hauschild (1970). Injection sites were evaluated after 24 hr.

Disc gel electrophoresis

Polyacrylamide gel electrophoresis was performed on the eluates from the columns, generally after the system of Davis (1964). A 4% gel, 5.0 × 60 mm, was used. Electrophoresis of the eluate was done initially at 1 ma/gel for 10 min and then at 3 ma/gel for 45 min. Gels were pre-run at 10 ma/gel for 30 min. The gels were stained with Coomassie Blue (0.025% in 7% acetic acid) and destained with 7% acetic acid.

RESULTS & DISCUSSION

CRUDE CELL EXTRACT from vegetative cells was used to produce antivegetative cell serum in rabbits. The IgG fraction when attached to Sepharose-4B produced a resin (V-resin) capable of binding antigens common to vegetative cells and sporulating cells. Figure 1 shows a typical elution pattern for the resin using 0.3 ml of sporulating cell extract. Following complete washing of the crude cell extract, antigens bound to the resin were removed using the same 0.01M phosphate buffer, pH 7.2, used for elution but with 0.2M NaCl added. Antigen-antibody complexes can be dissociated by a variety of means, but addition of NaCl was selected as least detrimental for the biologically active component. Higher sodium chloride concentration failed to remove additional material from the resins. Columns were stored at 5°C, but the isolation procedure was performed at room temperature to enhance antigen-antibody complex formation.

Both the unbound and bound fractions from the V-resin were concentrated and tested for erythemal activity in rabbits. Intradermal injections showed that the material not bound by the V-resin exhibited erythemal activity and that the bound fraction did not. These results demonstrated that the antigens common to vegetative and sporulating cells did not include the one responsible for erythemal activity in rabbits.

Subsequent passage of the toxic eluate from the V-resin through the column of S-resin resulted in the elution pattern shown in Figure 2. The material eluted with 0.2M NaCl in the buffer represented antigen bound to S-resin and was considerably less than that bound to the V-resin. Presumably, this was due to the presence of a smaller quantity of spore specific antigen in the cell extract. The effect of immobilization of the antibody on its affinity for the anitgen is speculative; however, it has been reported that chemical modification of protein by immobilization can cause shifts in pH optima, molecular stability, etc. The nature of the carrier might affect the strength of the interaction between the antigen and the insoluble antibody. Further, since antibodies combine with homologous antigen with varying degrees of avidity, adsorbed antibody may represent only a portion of the antibody population (Silman and Katchalski, 1966). Attachment of the antibody to the resin by interposing a hydrocarbon arm insures points of attachment only with α-amino groups (Cuatrecasas and Parikh, 1972). Removing the ligand from close proximity to the resin minimized steric interference during complex formation. In initial work, antibody attached directly to activated Sepharose-4B produced a resin drastically reduced in binding capacity. Direct attachment of ligand to resin is thought to produce multiple points of attachment, decreasing flexibility and mobility in the buffer.

Antigenic material bound to the S-resin was eluted with 0.2M NaCl in phosphate buffer, pH 7.2, concentrated and found to be erythemally active, characteristic of *C. perfringens* enterotoxin. The fraction not bound to the S-resin was also

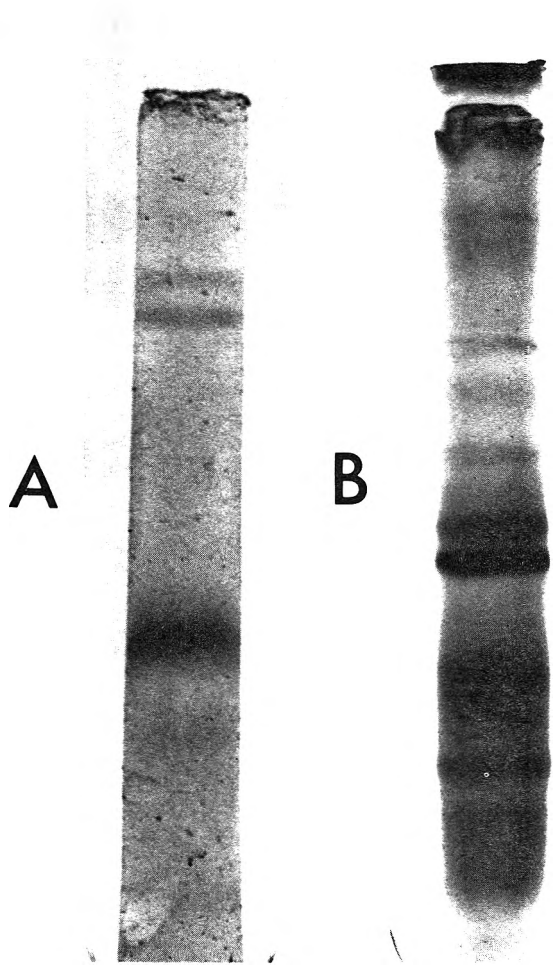

Fig. 3–Disc gel electrophoresis pattern of (A) toxin containing material bound by the S-resin; (B) crude sporulating cell extract.

active, due to having exceeded the binding capacity of the resin.

Disc gel electrophoresis resolved the S-resin bound antigens into five protein bands as determined by their reactivity with Coomassie Blue (Fig. 3a). Unstained gels were sectioned, extracted overnight in saline at 5°C, then injected separately and in combinations intradermally in rabbits to test for erythemal activity. It was found that only the slowest moving component, in Figure 3a, elicited erythemal activity. None of the other components separately or in combination invoked the erythemal response or enhanced erythema when combined with the toxic component. The toxin has been shown to be protein in nature (Hauschild and Hilsheimer, 1971), and we demonstrated it to be heat labile, nondialyzable and reactive with Coomassie Blue, confirming its protein nature.

Previous work has shown that ion exchange chromatography of the enterotoxin resulted in a partial conversion to two new, erythemally active forms (Hauschild and Hilsheimer, 1971). Such modification is unlikely by the procedure reported here as it commonly does not dissociate polymeric proteins.

Dissociation of the antigen-antibody complex by 0.2M sodium chloride was unexpected, as much harsher methods are commonly used; i.e., strongly acid or strongly alkaline conditions. It is possible that immobilization of antibody on resin had an effect on complex formation and stability. Derivatization of antibody through α-amino groups could bring about steric changes in the molecule affecting its antigen affinity. Retention of multiple components by the S-resin could be due in part to altered antigen-antibody specificity, or to the presence of a number of antigens unique to sporulating cells.

The binding capacity of the columns used in this procedure was found to be 102 μg of bound protein/ml of V-resin and 32 μg of bound protein/ml of S-resin. Figure 3 shows the disc gel electrophoresis pattern of: (a) toxin containing material bound by the S-resin; and (b) crude sporulating cell extract. The figure demonstrates a significant degree of purification achieved by the procedure. The erythemal activity of crude cell extract and of S-resin bound material was compared. A 150-fold purification was achieved based on the total amounts of protein required to elicit identical responses.

The immunoadsorbents used in this procedure could be recycled by washing with 0.01M phosphate, pH 7.2, to remove NaCl. Differences in binding specificity and capacity were not detectable with recycled resins. The procedure seems to be faster than other techniques since it required less than 24 hr for a complete run.

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Ms received 9/21/75; revised 12/13/75; accepted 12/16/75.

Presented at the 35th Annual Meeting of the Institute of Food Technologists, Chicago, Ill., June 8–12, 1975.

Published as Paper No. 3966, Journal Series, Nebraska Agricultural Experiment Station, Lincoln. Research reported was conducted under Project No. 16-22.

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STABILITY OF STAPHYLOCOCCAL ENTEROTOXIN A TO SELECTED CONDITIONS ENCOUNTERED IN FOODS

ABSTRACT

Changes in serological activity of enterotoxin A were measured by an immunodiffusion method. The reducing sugars xylose, lactose, glucose, maltose and fructose did not react with enterotoxin so as to reduce titer under various conditions of concentration, pH, temperature and time but all of these sugars exhibited an enterotoxin protective effect when systems were heated at 60°C. Sodium propionate, sodium benzoate and sodium nitrite at various concentrations did not alter enterotoxin titer when systems were incubated up to 9 days at 21 or 37°C. Sodium sulfite at a solution concentration of 1000 ppm decreased titers under specific conditions that suggest weak complex formation with the enterotoxin. Luxuriant growth of bacteria belonging to the genera *Bacillus, Micrococcus, Brevibacterium, Pseudomonas, Proteus, Escherichia, Enterobacter,* and yeasts of the genera *Candida* and *Saccharomyces,* in several media containing enterotoxin A did not significantly alter exterotoxin titer. Titer was substantially reduced by several species of lactic acid bacteria including *L. acidophilus, L. helveticus, L. plantarum, L. mesenteroides, Strep. thermophilus, Strep. faecalis* var. *liquefaciens, Strep. lactis* and *P. pentosaceus* in MRS broth and by five of the above species in APT broth. Reductions in titer were not simply due to decreases in pH by these organisms. Studies on heat inactivated enterotoxin revealed partial reactivation in phosphate-buffered saline incubated at 30 and 37°C for 72 hr but not at several other conditions of lower temperature and shorter time.

INTRODUCTION

MUCH RESEARCH on staphylococcal enterotoxins has been directed at their chemical and serological characterization, mechanisms of synthesis, antigenicity and pathology, production in bacteriological media and foods, extraction and purification, and methods for their assay, and this work has been well documented in reviews by Minor and Marth (1972a, b, c), Bergdoll and Robbins (1973), and Bergdoll et al. (1974). The effects of specific parameters such as nutrient supply, oxygen tension, incubation temperature, water activity, various salts, and associative growth of other microorganisms on growth and enterotoxin production by staphylococci have also been studied and reviewed (Tatini, 1973).

With respect to stability of preformed enterotoxin, reports have been relatively few, except in the area of thermal inactivation, where studies have been made on enterotoxin A (Chu et al., 1966; Denny et al., 1966, 1971; Hilker et al., 1968; Anon., 1973), enterotoxin B (Read and Bradshaw, 1966a, b; Satterlee and Kraft, 1969; Jamlang et al., 1971; Fung et al., 1973), enterotoxin C_1 (Fung et al., 1973), and enterotoxin D (Anon., 1973). Bergdoll (1970) and others further reported that enterotoxins, in an active state, are quite resistant to the proteolytic enzymes trypsin, chymotrypsin, rennin, papain, and pepsin. Lee et al. (1975) were able to detect enterotoxins A and C in a dried pasta after 1 yr at 25°C. On the other hand, Donnelly et al. (1968) observed that enterotoxin A produced in milk disappeared from some samples on prolonged incubation at 35°C.

There is need for more information on the fate of enterotoxin under conditions which may be encountered in foods, particularly enterotoxin A which is most commonly implicated in human food poisoning outbreaks. This is especially so since there is a growing tendency to combine different ingredients into analogs and manufactured foods with the possibility that some of these ingredients might contribute enterotoxin to final products not commonly associated with the growth of *S. aureus.* The present study was undertaken to determine effects of reducing sugars and selected antimicrobial food additives on stability of enterotoxin A, to investigate the effects of several food-related microorganisms on stability of enterotoxin A, and to examine its possible reactivation following partial thermal inactivation.

EXPERIMENTAL

Enterotoxin A, antiserum and assay procedure

Lyophilized crude enterotoxin A and its specific antiserum were obtained from the Food Research Institute, University of Wisconsin, Madison. A pH 7.4, 0.02M phosphate-buffered saline diluent (PBS) containing 0.85% NaCl and 0.01% merthiolate (Weirether et al., 1966) was used in preparing all antiserum agar and as an enterotoxin suspending medium in some of the experiments to be described. Phosphate buffer solutions (pH 7.4 and 8.1) without saline and merthiolate were used as enterotoxin suspending media in the reducing sugar studies. Where the effects of microbial growth upon enterotoxin stability were investigated, enterotoxin was suspended in the specific medium selected for optimum growth of each organism.

The Oudin single gel-diffusion tube test, as modified by Weirether et al. (1966), was used as the method for enterotoxin A assay throughout the present study. Pyrex glass tubes having an inside diameter of 4 mm were internally precoated with a 0.6% solution of Noble agar (Difco) in PBS, which upon solidifying prevented enterotoxin from channeling between the antiserum agar and the capillary wall. Antiserum agar was prepared by mixing equal volumes of tempered 0.6% Nobel agar in PBS with diluted antiserum in PBS to give an antiserum titer of 1:38. Coated assay tubes were filled to a height of 5 cm with antiserum agar which was permitted to solidify in an upright position and then overlayered with an equal volume of enterotoxin suspension. Tubes were then incubated upright at 37°C for 72 hr prior to measurement of precipitin bands. The method's lower threshold of enterotoxin sensitivity (1 μg/ml) was considered adequate since initial concentrations of enterotoxin used in the experiments to produce readily measurable bands were never below 10 μg/ml. Standard curves for enterotoxin in all suspending media were constructed by graphing known concentrations of enterotoxin against observed precipitin band widths on semilogarithmic graph paper using the method of least squares. In all cases straight-line responses between enterotoxin concentration and band width were obtained and it was determined that differences of 1.0 mm or greater in precipitin band widths between treated and control samples of enterotoxin could be readily duplicated and such differences were considered significant. In subsequent tables all enterotoxin titers are reported as μg/ml of suspending medium from corresponding standard curves.

Interactions with reducing sugars

Possible Maillard type complexing between enterotoxin A and several reducing sugars was investigated by preparing solutions of xylose, lactose, glucose, maltose, and fructose in pH 7.4 and 8.1 phosphate buffers. One ml quantities of these solutions in tubes were mixed with an equal volume of enterotoxin in the buffers to give final concentrations of 10 μg/ml enterotoxin A and 1.25 and 5.0% reducing sugars.

Tubes were then sealed and heated at 60°C for 20, 40, 60, 80, and 100 min and at 45°C for 90 and 180 min to promote possible Maillard complexing. In all cases timing was not begun until tubes reached 60°C or 45°C. After heating tubes were immediately chilled in ice water and analyzed for enterotoxin titer.

Interaction with antimicrobial food additives

Solutions of sodium propionate ("Mycoban," Pfizer, Inc.), sodium benzoate (Pfizer, Inc.), sodium nitrite (Mallinckrodt-AR), and sodium sulfite (B & A, Allied Chemical) in PBS were prepared and 1.0 ml quantities of each were added to separate tubes and mixed with an equal volume of enterotoxin in PBS containing 5.0% peptone (Bacto-Peptone, Difco) to yield final concentrations of 1.0, 0.1 and 0.01% sodium propionate or sodium benzoate, 500, 50 and 5 ppm sodium nitrite, 1000, 100 and 10 ppm sodium sulfite, 10 μg/ml enterotoxin A, and 2.5% peptone. The peptone was included to introduce organic material and more closely approximate a food system. Tubes were sealed and duplicate sets incubated for 1, 3, 6 and 9 days at 21 and 37°C, after which enterotoxin titers were determined. In no case did initial system pH vary more than ± 0.1 pH unit from the enterotoxin system without additives, which was lowered to 7.1 by the peptone.

Microbial growth effects on stability of enterotoxin

Microorganisms were obtained from the Cornell University Culture Collection and included the proteolytic bacteria *B. cereus* (strains T, A.T.C.C. no 6464,7004,14579), *B. pumilus, B. subtilis, M. caseolyticus, B. linens, Ps. fragi, Ps. fluorescens, P. vulgaris,* the enteric bacteria *E. coli, E. coli* (rat intestine isolate), *E. aerogenes, E. aerogenes* (rat intestine isolate), *Strep. faecalis* var. zymogenes (rat intestine isolate), the lactic acid bacteria *L. acidophilus, L. helveticus, P. pentosaceus, Strep. thermophilus, Strep. faecalis* var. *liquefaciens, L. plantarum, L. mesenteroides, Strep. lactis,* and the yeasts *C. utilus, C. lipolytica, Sac. cerevisiae, Sac. fragilis,* and *Sac. lactis.* Proteolytic bacteria were inoculated into 2 ml volumes of Nutrient broth pH 6.9 (BBL), Brain Heart Infusion broth pH 7.3 (BBL), and Trypticase Soy broth pH 7.2 (BBL); enteric bacteria into Brain Heart Infusion and Trypticase Soy broths; and the yeasts into APT pH 6.4 (BBL) and MRS pH 6.0 (Oxoid) broths all containing 20 μg/ml of enterotoxin A. Lactic acid bacteria were inoculated into 10 ml volumes of APT and MRS broths each containing 10 μg/ml of enterotoxin. All cultures were agitated and then incubated at 30 or 37°C, depending on the organism. At 2- and 5-day intervals cultures were again agitated and then centrifuged for 10 min in a Safeguard clinical centrifuge with 13 cm conical head (Clay-Adams, Inc., New York, N.Y.) at 3000 rpm (speed 6) after which a sample of the supernatant was analyzed for enterotoxin titer and pH of the remaining material determined. Prior to analysis of lactic cultures the reduced pH's of these samples were neutralized with 10N NaOH since preliminary investigations indicated that low pH produced substantial clouding in the serum-agar of assay tubes making detection of precipitin bands impossible.

Thermal inactivation and reactivation of enterotoxin

Sets of tubes were prepared with half containing 10 μg/ml of enterotoxin in PBS and the other half containing 10 μg/ml enterotoxin in PBS plus 2.5% peptone. The peptone was included to provide amino acids for possible use in reconstruction of damaged enterotoxin. All tubes were sealed and heated to 60°C, except for unheated controls, and then held for periods up to 100 min to effectuate progressive degrees of enterotoxin inactivation. After heating, tubes were immediately chilled and those not incubated for reactivation were analyzed for enterotoxin titer. Other sets were incubated at 4, 22, 30, and 37°C for 24, 48, and 72 hr, gently agitated and analyzed for enterotoxin titer. Measurements revealed pH values of 7.4 and 7.1, respectively, for the PBS plus enterotoxin and the PBS plus peptone plus enterotoxin systems.

RESULTS & DISCUSSION

THE EFFECTS of glucose on enterotoxin A titer under varying conditions of concentration, pH, temperature and time are presented in Table 1. These data are representative of those obtained with xylose, lactose, maltose, and fructose under the same conditions. There was no decrease in enterotoxin titer relative to control samples without sugar for any of the reducing sugars at any concentration, pH, temperature, or time investigated. While there was no evidence of enterotoxin heat inactivation in any of the systems exposed to 45°C for periods up to 180 min, heat inactivation did occur at 60°C and all reducing sugars exhibited an enterotoxin protective effect at 60°C both at pH 7.4 and 8.1. The degree of protection generally was dependent upon reducing sugar concentration, with 5.0% being more effective than 1.25%. If in fact a Maillard complexing did occur between the enterotoxin and any of the reducing sugars, it did not appear to alter its antigenicity as determined by the assay procedure employed.

Although some food additives including $NaNO_3$ and $NaNO_2$ have been present in foods investigated for their abilities to support growth of *S. aureus* and enterotoxin production (Tatini, 1973), specific food additives have not been studied for their effects upon the stability of preformed enterotoxin A. In the present investigation sodium propionate and sodium benzoate at levels of 0.01, 0.10 and 1.0% and sodium nitrite at levels of 5, 50 and 500 ppm in PBS plus 2.5% peptone did not alter enterotoxin A titer when samples were incubated for 1–9 days at either 21 or 37°C. However, 1000 ppm sodium sulfite reduced enterotoxin titers to a nondetectable level in samples incubated up to 9 days at 21°C and for 1 day or less at 37°C. Samples incubated with 1000 ppm sodium sulfite for 3, 6 and 9 days at 37°C possessed enterotoxin titers equal to controls without sodium sulfite as did all samples containing 10 and 100 ppm sodium sulfite. A possible explanation for these observations might be formation of a weak complex between sulfite or bisulfite ions and enterotoxin A molecules at low temperatures (21°C) that interferes with their detection by the assay procedure, and instability of the complex at higher temperatures (37°C) for prolonged periods, releasing the enterotoxin molecules with no apparent alteration in their serological activity. Since 1000 ppm sodium sulfite is above the levels commonly added to certain foods, further study of lower levels upon enterotoxin stability appears warranted.

The effects of microbial growth upon enterotoxin A titer in different media are presented in Table 2. Luxuriant growth of the proteolytic bacteria did not significantly alter enterotoxin titers during incubation for 2 and 5 days in Brain Heart Infusion, Trypticase Soy, and Nutrient broths initially containing 20 μg/ml of enterotoxin. The slight increase in titer of uninoculated control samples in nutrient broth after 5 days may have been due to some reactivation of previously damaged enterotoxin molecules in this medium. The enteric bacteria did not significantly alter the same initial enterotoxin titer in Brain Heart Infusion or Trypticase Soy broths nor did the yeasts in APT or MRS broths after 2 or 5 days incubation. Growth of all lactic acid bacteria resulted in marked reductions in pH of the APT and MRS broths initially containing 10 μg/ml of enterotoxin, and in numerous cases, after neutralization of these broths, substantial reductions in enterotoxin titer compared to uninoculated controls were noted. Significant reductions in titer occurred with *L. acidophilus, L. helveticus, P. pentosaceus, Strep. thermophilus,* and *Strep. faecalis*

Table 1—Effects of glucose on enterotoxin A titer under varying conditions

Glucose conc (%)	Buffer pH	Enterotoxin titer (μg/ml) Min at 60°C 0	20	40	60	80	100	Min at 45°C 0	90	180
0	7.4	10	3	2	<1	<1	<1	10	10	10
1.25	7.4	10	6	4	3	2	2	10	9	10
5.0	7.4	10	8	6	4	3	3	10	10	10
0	8.1	10	5	2	2	<1	<1	10	10	10
1.25	8.1	10	8	5	4	3	3	10	10	10
5.0	8.1	10	9	8	6	5	4	10	10	10

Table 2—Effects of microbial growth on enterotoxin A titer in different media

Organism	Temp (°C)	Nutrient broth 2 days µg/ml	Nutrient broth 2 days pH	Nutrient broth 5 days µg/ml	Nutrient broth 5 days pH	Brain heart infusion broth 2 days µg/ml	Brain heart infusion broth 2 days pH	Brain heart infusion broth 5 days µg/ml	Brain heart infusion broth 5 days pH	Trypticase soy broth 2 days µg/ml	Trypticase soy broth 2 days pH	Trypticase soy broth 5 days µg/ml	Trypticase soy broth 5 days pH
None	30	20	6.9	24	7.2	20	7.3	20	7.3	20	7.3	19	7.2
None	37	20	7.0	28	6.9	20	7.3	20	7.2	20	7.3	20	7.2
B. cereus (T)	30	20	7.3	20	8.1	20	6.1	20	7.7	19	6.0	19	7.2
B. cereus (6464)	30	20	7.7	20	8.2	20	6.3	20	7.4	19	5.9	19	7.5
B. cereus (7004)	30	20	7.7	17	8.6	20	6.0	18	7.5	19	5.8	19	7.8
B. cereus (14579)	30	20	7.2	20	8.1	20	6.2	20	6.9	19	5.7	19	6.7
B. pumilus	30	17	7.6	17	8.2	20	6.2	18	7.4	19	6.4	16	8.0
B. subtilis	30	20	7.6	20	8.3	20	7.0	20	7.9	20	6.2	19	7.9
M. caseolyticus	30	NG[a]	NG	20	7.1	20	6.7	18	5.9	20	6.7	19	5.8
B. linens	30					20	7.4	20	7.6	19	7.4	19	7.6
Ps. fragi	30	20	7.9	20	8.3	20	6.8	20	8.4	20	7.6	19	8.0
Ps. fluorescens	30	20	8.0	20	8.5	20	7.9	20	8.3	19	7.7	19	8.1
P. vulgaris	37	20	8.4	20	8.5	20	7.4	20	8.4	20	6.6	19	6.7
E. coli	37					20	6.9	20	8.3	20	5.5	20	5.9
E. coli (rat)	37					20	7.4	20	8.6	20	5.6	20	8.1
E. aerogenes	37					20	7.7	20	8.9	20	7.7	20	8.7
E. aerogenes (rat)	37					20	7.2	20	8.4	20	6.9	20	8.2
Strep. zymogenes (rat)	37					20	5.8	18	5.8	20	5.6	19	5.3

Organism	Temp (°C)	APT broth 2 days µg/ml	APT broth 2 days pH	APT broth 5 days µg/ml	APT broth 5 days pH	MRS broth 2 days µg/ml	MRS broth 2 days pH	MRS broth 5 days µg/ml	MRS broth 5 days pH
None	30	18	6.4	16	6.4	20	6.1	20	6.1
C. utilus	30	18	6.3	18	6.3	20	5.7	20	5.9
C. lipolytica	30	18	7.2	16	7.8	20	6.7	17	7.9
Sac. cerevisiae	30	18	6.3	18	6.2	20	5.9	20	5.8
Sac. fragilis	30	18	6.3	20	6.3	20	5.9	20	5.9
Sac. lactis	30	18	6.3	18	6.3	20	5.9	20	6.0
None	30	10	6.3	9	6.3	10	6.1	10	6.1
None	37	10	6.3	5	6.3	10	6.1	10	6.1
L. acidophilus	37	1	4.2	1	4.3	2	3.6	2	3.4
L. helveticus	37	1	4.2	<1	4.3	1	4.1	1	4.1
P. pentosaceus	37	2	4.3	<1	4.3	<1	4.0	<1	3.9
Strep. thermophilus	37	4	4.4	3	4.5	2	4.3	<1	2.3
Strep. liquefaciens	37	5	4.5	3	4.6	<1	4.5	1	4.6
L. plantarum	30	10	4.5	8	4.6	2	3.6	2	3.6
L. mesenteroides	30	10	4.8	9	4.9	2	4.2	2	4.1
Strep. lactis	30	10	4.3	6	4.4	4	4.2	2	4.3

[a] NG indicates no growth of test organism

var. *liquefaciens* when incubated in APT broth, and with all lactic acid bacteria investigated in MRS broth, within 2 days of incubation. *Lactobacillus plantarum, L. mesenteroides,* and *Strep. lactis* did not markedly alter enterotoxin titer in APT broth during either 2 or 5 days of incubation, even though pH values in these cultures were reduced to levels comparable to systems demonstrating substantial reductions in titer. These results indicate that the decreases in enterotoxin titer produced by the lactic acid bacteria were not due simply to the acid produced but could be due to specific enzymes or other metabolites elaborated in the different media acting alone or in combination with high acidity. Further experimentation in which quantities of APT and MRS broths containing enterotoxin but without microbial inoculation were adjusted to pH values of 3, 4, 5 and 6 with lactic acid and then incubated at 37°C for 2 hr, after which samples were neutralized with 10N NaOH and analyzed for enterotoxin titer, tend to support the above conclusion since reduced pH under these conditions had little or no effect upon enterotoxin titer. Other possibilities for the decreased titers include physical adsorption of toxin to bacterial cells and its removal along with cells in obtaining supernatants for toxin assay, and effects of specific metabolites on the antigen-antibody reaction. The former possibility would require that such adsorption be quite specific since titers were not reduced by many of the organisms tested nor was titer reduced in APT broth by three lactic acid organisms that were effective in MRS broth. Effects of specific metabolites on the antigen-antibody reaction were not investigated in the present study.

Fung et al. (1973) have reported on the reactivation of partially thermally inactivated enterotoxins B and C_1. However, no such investigations have been reported concerning enterotoxin A. The thermal inactivation and reactivation of enterotoxin A in PBS without peptone is presented in Table 3.

Table 3—Reactivation of enterotoxin A following heating at 60°C in PBS

Reactivation conditions		Enterotoxin titer (μg/ml)[a]										
		Min at 60°C										
Temp (°C)	Time (hr)	0	10	20	30	40	50	60	70	80	90	100
Control	0	10	8	6	5	4	4	3	3	3	<3	<3
4	24	10	8	7	6	4	4	3	3	3	3	<3
4	48	10	8	7	5	4	4	3	3	3	3	<3
4	72	10	8	7	5	4	4	3	3	3	3	<3
22	24	11	8	7	6	5	4	3	3	3	3	<3
22	48	11	10	8	5	4	4	3	3	3	3	3
22	72	10	8	8	5	4	4	3	3	3	3	3
30	24	11	8	7	6	4	4	4	3	3	3	<3
30	48	11	8	8	6	4	4	3	3	3	3	3
30	72	13	11	8	8	6	4	4	4	4	4	4
37	24	11	8	7	6	5	4	4	4	3	3	<3
37	48	11	10	8	6	6	5	4	3	3	3	3
37	72	15	15	11	8	8	8	6	5	5	6	5

[a] In PBS a titer of 3 μg/ml corresponds to a precipitin band of 0.5 mm, the smallest band that could be readily detected.

Thermal inactivation proceeded at a faster rate and to a greater degree in PBS without peptone than in identical systems with peptone. No reactivation of enterotoxin A from control titers was noted in any of the samples containing 2.5% peptone nor in any of the samples without peptone incubated at 4 and 22°C. However, partial reactivation took place in heated samples without peptone incubated at 30 and 37°C for 72 hr. Indeed, even in the unheated samples incubated at 30 and 37°C for 72 hr some increase in enterotoxin titer occurred. A possible explanation for this may be the presence of some partially denatured enterotoxin molecules in the source material which tend to reactivate when held at elevated temperatures.

In summary, these studies provide further evidence for the high degree of stability of staphylococcal enterotoxin A to a variety of stresses that may be encountered in foods. The results indicate that enterotoxin A activity may actually be somewhat protected by reducing sugars and possibly other organic materials and that some reactivation of partially thermally inactivated enterotoxin A can occur on storage. The inactivation of enterotoxin A by several lactic acid bacteria but not by acid alone nor numerous other organisms suggests the involvement of specific enzymes or other metabolites.

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Ms received 10/24/75; revised 12/19/75; accepted 12/25/75.

The authors thank Dr. M.S. Bergdoll and Mrs. R.N. Robbins (Food Research Institute, Univ. of Wisconsin, Madison) for supplying the enterotoxin A and antiserum and for technical advise during the present study.

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WATER ACTIVITY DETERMINATION: A COLLABORATIVE STUDY OF DIFFERENT METHODS

ABSTRACT

Seven methods of water activity (a_w) measurement were tested in a collaborative study between three laboratories. These methods included the direct measurement of vapor pressure, an isopiestic technique and several electronic devices. Both saturated salt solutions and foods were measured. When compared to standards, the vapor pressure manometric (VPM) technique gave the best results. However, the absolute values of the standards are questionable. The results of the comparison of the values for foods showed a range of ±0.02 a_w units. Thus, one can question the validity of literature values reported to three decimal places or the absolute values for limits on microbial growth. This research suggests that some standards must be set for all research groups and shows that overall, the VPM is probably the best and most accurate method.

INTRODUCTION

WATER ACTIVITY (a_w) is an important property in the manufacture of food systems and formulations. Most chemical reactions and microbiological activity are controlled directly by the water activity of the food system as reviewed by Labuza (1974). This study was undertaken because of the lack of a standard method of measuring a_w which would ensure comparable results for different investigators. At present, most investigators have their own method of a_w determination, and in some cases report a_w to three decimal places. It is not known definitely if the method used is that accurate or which method is most precise.

This study has involved the preparation of various saturated salt solutions, commercial foods and model food systems by our laboratory at the University of Minnesota, and the determination of the sample a_w's by us as well as by Dr. J. Flink at Massachusetts Institute of Technology and W. McCall of Armour and Co. Seven techniques or instruments were employed. The methods of a_w determination included in this study were:

1. Brady Array
2. Humichek
3. Relative humidity indicator 400D
4. Hygrodynamics Hygrometer
5. Sina-scope
6. Equilibrium moisture adsorption (Fett-Vos method)
7. Vapor pressure manometer (VPM)

Saturated salt solutions are usually used for calibration in most methods, so a wide range was used. With but few exceptions, all samples have been analyzed in duplicate by the seven methods of a_w determination. The following points were considered in this study:

1. Did the measured value agree with the theoretical literature value (in the case of saturated salt solutions)?
2. Did the results of the various methods show good correlation with each other?
3. Was there evidence that volatile glycols, or other properties of the samples, caused interference with the a_w determination?
4. Did any single method seem outstandingly better over the full a_w range for foods or saturated salt slurries?

MATERIALS & METHODS

THE SATURATED SALT SOLUTIONS tested in the study were $MgCl_2$, $Mg(NO_3)_2$, $(NH_4)_2SO_4$, $CdCl_2$, Li_2SO_4, Na_2HPO_4, and K_2SO_4. The salts (Analytical Reagent Grade, AR) were obtained from the University of Minnesota Chemical Storehouse. Saturated solutions (slurries) of these salts were made with distilled-deionized water according to the method described in Hygrodynamics Bulletin No. 5. One large bottle of each slurry served as a source for all of the determinations. However, during the course of the study a question arose as to the purity of the stock slurry of Li_2SO_4 and a small amount of fresh slurry was made to check the results. The two batches had the same a_w as measured by the hygrometer and by the VPM so there was confidence in the purity of the salt.

Aliquots of these samples were decanted into glass 4 oz jars and covered with parafilm and plastic screw caps. The samples were held at 22°C (room temperature) until sent out for analysis by the various other methods. It has been stressed that the subsamples came from one source because this study involves reproducibility in a_w determination, not in the preparation of a saturated salt slurry per se. It must be assumed that if AR grade salts and "pure" water are used, the a_w of a slurry is consistent for any particular salt.

An exception to the above assumption occurs, however, when the a_w of the slurry is high enough for microbial growth. If an autotrophic bacteria is present, and it can grow on Na^+, H^+ and $PO_4^{\equiv}$, then the a_w of that slurry may change. In fact, this occurred in a sample of the Na_2HPO_4 slurry in which microbial activity was detected while measuring on the VPM since it showed a continuous increase in pressure due to gas evolution. For this reason, the VPM cannot be used on samples supporting microbial activity. The results of other methods of determination would not be affected by this gas evolution from microbial growth.

The foods and model food systems that were analyzed in the study (Table 1) were chosen to represent a wide a_w range; high moisture, intermediate moisture and dry foods. The thuringer had been fermented, smoked and cooked according to sausage manufacturing conditions. The cheese, bread and intermediate moisture cat food were purchased at a local supermarket. Commercial IM cat food represented a food high in glycols. Hennican, an intermediate moisture model food system, was made according to the composition described by Acott and Labuza (1975). K-sorbate (0.3%) was added to retard spoilage during handling. Two other Hennican systems containing glycols were also tested. The semolina-egg dough was made using Como No. 1 semolina (Capital Duram Div., International Multifoods Corp., Minneapolis) plus 5.5% whole egg solids (A.J. Pietrus and Sons, Co., Sleepy Eye, Minn.). Protein hydrolyzate powder (PHP, Mead Johnson, Evansville, Ind.) and NZ-amine (Sheffield Chemical, Kraftco Corp., Oneonia, N.Y.) systems were made with distilled water as described by Troller (1971). The theoretical a_w of these systems, 0.90 and 0.93, as reported by Troller was based on using a calibrated hygrometer sensor. A commercial dry soup mix was chosen as a typical dry product. The sausage and protein-

based bacteriological media were prepared by Mr. Robert Lee and Dr. Sita R. Tatini of the Dept. of Food Science & Nutrition, University of Minnesota.

Preparation and handling of samples

The saturated salt slurries were stored in 4 oz jars covered by parafilm and a plastic screw cap. The salts were held at room temperature and equilibrated at the temperature of analysis for 24 hr prior to analysis. It is important to note that these were salt slurries. They consisted of a large excess of crystalline salt in a saturated solution which was just sufficient in volume to cover the salt crystals.

In general, room temperature storage of salt slurries is sufficient. Those of $a_w \geq 0.97$ should be stored at 4°C to prevent microbial activity or made up fresh and held at room temperature for only a limited amount of time. If room temperature storage were preferred, the possibility of contamination by microbes could be reduced by using sterile water and glassware, and aseptic techniques. Slurries of $a_w \geq 0.97$ made and stored in this way should still be examined closely for microbial activity as the salt crystals themselves may carry some microbial contaminants.

The foods and model food systems were packed in 202 × 214 epoxy-lined tin cans. The loaves of the commercial white bread, packages of the individually wrapped slices of American processed cheese, the soup mix and the cat food were purchased on the day prior to canning. Nine slices of bread were stacked up and the can was used to stamp out the centers of the slices. No crust was used. The compacted bread was then sealed in the cans. The cheese was unwrapped, cut into 1 cm strips and tightly stacked into the cans, leaving little air space. The cat food was ground in a blender to ensure a homogeneous sample. The soup mix was tested as it came from the manufacturer's pouch. The cans were sealed immediately. The Hennican systems and semolina-egg dough was also packed into cans and sealed. In all cases the food was at room temperature at the time of canning and sealing.

Precautions were taken to keep microbial contamination to a minimum. The cans were sanitized with 500 ppm hypochlorite solution. Sterile gloves were worn during handling of the cheese and bread. Sterile wooden blades were used to transfer the Hennican.

The bacteriological media, NZ-amine–PHP, was made in two batches, one at each a_w. After mixing and solubilizing all the protein the batches were poured into four 250 ml Nalgene bottles with screw caps. The bottles were filled half-full to leave space for the foaming which occurred during autoclaving. The samples were autoclaved for 15 min and then brought to atmospheric pressure very slowly. The sterile media was cooled to room temperature; then the caps were tightened.

Instructions on sample handling were sent to investigators prior to sending the samples. The salts were held at room temperature, the perishable foods were kept at 4°C (except for the 24 hr pre-analysis temperature equilibration), and the sterile media were kept at room temperature and not transferred or opened until just prior to analysis. Samples were prepared and sent on two separate occasions to the other investigators. In some cases due to instrument problems, not all samples were tested by each method as indicated in the results by "not tested."

The analyses were performed at 21–23°C. The food samples were transferred into 4 oz snap cap vials 24 hr prior to analysis. If a hygrometer was used the sensor mounted in a cover for the vial was fixed over the sample, replacing the original cover and forming an air-tight seal. The procedure from this point was performed as necessary for each instrument.

Measurement

The values for the standards (saturated salt solutions) used to calibrate the instruments were established individually by each investigator taking an average of values found in the literature. The standard salt solutions and references used by M.I.T. appear in Table 2 and those used by the University of Minnesota in Table 3. The measurements at Armour were only made on the Sina-scope instrument and the instrument was calibrated as will be described later. Table 4 lists a comparison of the average values for the salts from Tables 2 and 3. The average value from the M.I.T. data was obtained by averaging the 20° and 25°C data from references 1 through 8 of Table 2 while only the 20° data from Table 3 were used for comparison. The standard values were the same in all cases except for three salts, $KC_2H_3O_2$, $Ca(NO_3)_2$, and KNO_3 where a 0.01–0.02 deviation was seen. Since most values are the same, the results from M.I.T. should be directly comparable with the University of Minnesota results; however, this shows the problem in relying on literature values and averages in preparing calibrations.

The Brady Array (Thunder Scientific Co., Albequerque, New Mexico) is a bulk-effect device. The manufacturer suggests that this mode of sensor equilibration is much faster than the surface type devices. The interaction occurs within the structure of the sensor. It consists of a precise array of crystal semiconductor junctions and spaces. The presence of water molecules imposes stress on the bonds of the crystal lattice structure. As the stress increases, the bonds become distorted. Energy is released to the free electrons within the structure effecting an increase in conductivity. The change in conductivity is used as a measure of the concentration of water molecules in the lattice, and this is related via an electrical signal to % relative humidity (Bennewitz, 1973).

Table 1—Foods analyzed in study

Thuringer sausage
Processed American cheese (Kraft) — individual wrapped slices
Bread (Wonder Bread) — sandwich type
IM Cat food (Tabby)
Hennican (a_w 0.91)
Hennican plus 2% glycerol
Hennican plus 2% 1,3-butylene glycol and 2% propylene glycol
Semolina dough mix + 5.5% whole egg solids
Microbiological growth media, NZ-amine and protein hydrolysate, 0.90 (Troller, 1971).
Microbiological growth media, NZ-amine and protein hydrolysate, 0.93 (Troller, 1971).
Dry soup mix (Lipton vegetable soup)

The Brady Array system as used by M.I.T. in this study consisted of a BR-101R Brady Array connected by cable (C-3A) to a signal conditioning module (SC-1020M). Voltage output was read on a digital output device. A strip chart recorder (Heath IR-18M) simultaneously produced a graph which showed when vapor space equilibrium had been reached. Although this system has been claimed to give low hysteresis effects (Bennewitz, 1973), severe hysteresis was experienced. Furthermore, there is no direct correlation of voltage output with known a_w values. Because of this, constant standardization of the instrument was necessary.

The procedure for the Brady Arnay was as follows:

1. Determine the recorder reading which corresponds to the theoretical a_w of a primary standard salt solution. All measurements were in the adsorption direction, i.e., the salt of theoretical a_w below that expected for the driest sample, was tested first. The sensor was sealed over the sample. The recorder results showed that the a_w values after 20 or 30 min had not changed significantly, therefore, the 30 min reading was used. The theoretical a_w of the standard salt solution was taken from the M.I.T. standard value in Table 2. The recorder value for the standard was plotted on a graph vs a_w.

2. Determine the recorder reading that results from 30 min exposure of the sensor to the unknown sample. No desiccation of the sensor was done between these samples.

3. Determine the 30 min recorder reading that results from a standard salt solution of an a_w near, but above, that expected for the unknown. Plot this value vs a_w and draw a line connecting the values for the two known salts. Interpolate the recorder reading of the unknown and read off the a_w on this line.

4. Determinations of samples of higher a_w were done then, continuing in the adsorption mode and interrupting every 1 or 2 samples by a standard salt solution.

5. When samples of lower a_w were subsequently analyzed, the sensor was held over drierite until the recorder value showed desorption to a_w 0.0, which took about ½ to 1 hr.

6. When not in use, the sensor was stored over drierite.

A plot of the recorder output vs a_w values of standard salt slurries was not reproducible from day to day. Thus, the interpolation method described above was found to be necessary for every determination. The range of best sensitivity was found to be from 0.4–0.8 a_w because of a sigmoid curve that results between a_w and voltage. The manufacturers claim that signal conditioning can create a curve that is linear from 0–1.0 a_w. The National Bureau of Standards has published a report (#NBSIR-74-477) on the Brady Array which mentions problems including those found in this study.

The Humichek (Beckman Instruments, Cedar Grove, NJ) is an electric hygrometer and was tested at M.I.T. The sensors for the instrument used in this study were modified so that they could be used with the 4 oz snap cap vials. The sensor was remounted in a 9-pin miniature tube socket into a #7 rubber stopper. Although very little hysteresis was experienced, samples were always tested in an adsorption mode.

The procedure for the Humichek was as follows:

1. The sensor was inserted into the vial forming an air-tight seal.

2. The instrument thumbwheel was adjusted to balance the bridge signal. Two LED lamps indicated high and low reading. The thumbwheel was calibrated so that a direct reading of % relative humidity

Table 2—Water activity values from the literature (M.I.T.)[a]

Salt	(1) 22°C	(2) 20°C	(3) 22°C	(4) 20°C	(5) 25°C	(6) 25°C	(7) 20°C	(8) 20°C	(9) 20°C	(10) M.I.T. Standard
$KC_2H_3O_2$	0.230	0.23	0.215	0.200	0.225*	0.227*	–	–	0.20	0.23
$MgCl_2$	0.33	0.33	0.325	0.340	0.330*		0.336	–	0.33	0.33
				0.331*		0.324*	0.332*			
$Zn(NO_3)_2$	–	0.38	–	0.420	–	–	–	–	0.42	0.40
$Ca(NO_3)_2$	0.522	0.56	0.507	0.560	–	–	–	–	–	0.54
				0.558*						
$Na_2Cr_2O_7$	0.582	–	–	0.520	–	0.536*	0.552	0.542*	0.52	0.54
							0.538*			
$NaNO_2$	0.648	–	–	0.660	–	–	–	0.630**	0.66	0.66
NaCl	0.756	0.75	–	0.765		–	0.755	0.757	0.76	0.76
				0.758*	0.753*				0.75**	
$(NH_4)_2SO_4$	0.802	0.79	–	0.817	–	–	0.806	–	0.81	0.81
				0.810*					0.81**	
KNO_3	–	0.94	0.920	0.942		–	0.932	–	0.94	0.93
				0.930*	0.925*		0.920*		0.93**	

[a] (1) Wink and Sears (1950)
(2) Rockland (1960)
(3) Thunder data sheet on Calibration Cells
(4) O'Brien (1948)
(5) Stokes and Robinson (1949)
(6) Richardson and Malthus (1955)
(7) Wexler and Hasegawa (1954)
(8) Carr and Harris (1949)
(9) International Critical Tables (1926)
(10) Average of data from ref 1–8 (20 and 25° C data)
*25° C; ** 30° C

Table 3—Water activity values for saturated salt solutions from the literature (Univ. of Minnesota)

Saturated salt solutions	°C	Int'l Crit. Tables (1926)	Wexler & Hasegawa (1954)	Rockland (1960)	Handbook of Chem. Phys. (1972–73)	Avg Value	U. of Minn. Standard
$LiCl \cdot H_2O$	20	0.15	0.124	0.12	0.15	0.14	–
	25	–	0.120	0.11	–	0.12	
$KC_2H_3O_2$	20	0.20	–	0.23	0.20	0.21	0.27
	25	–	–	0.23	–	0.23	
$MgCl_2 \cdot 6H_2O$	20	0.33	0.336	0.33	–	0.33	0.33
	25	0.32	0.332	0.33	–	0.33	
KCO_3	20	–	–	0.44	0.44	0.44	0.44
	25	–	–	0.43	0.43	0.43	
$Mg(NO_3)_2 \cdot 6H_2O$	20	0.55	0.549	0.52	–	0.54	0.53
	25	0.52	0.534	0.52	0.52	0.52	
NaCl	20	0.76	0.755	0.75	–	0.76	0.76
	25	0.75	0.758	0.75	–	0.75	
$(NH_4)_2SO_4$	20	0.81	0.806	0.79	0.81	0.81	0.81
	25	0.81	0.803	0.79	0.81	0.80	
$CdCl_2$	20	–	–	0.82	–	0.82	0.82
	25	–	–	0.82	–	0.82	
Li_2SO_4	20	–	–	0.85	–	0.85	–
	25	–	–	0.85	–	0.85	
K_2CrO_4	20	0.88	–	0.88	0.88	0.88	–
	25	–	–	0.87	–	0.87	
KNO_3	20	0.94	0.932	0.94	–	0.94	0.94
	25	0.93	0.920	0.93	–	0.93	
Na_2HPO_4	20	0.95	–	0.98	0.95	0.96	0.95
	25	–	–	0.97	–	0.97	
K_2SO_4	20	0.97	0.972	0.97	–	0.97	0.97
	25	0.97	0.969	0.97	–	0.97	

Table 4—Change in calibration for the Hygrodynamics Hygrometer

Sensor	Sensor number	Calibration salt	Theoretical a_w of salt slurry[a]	Correction factor for sensor	Check on correction factor	Difference
Red	405481	$KC_2H_3O_2$	0.21	+0.08	+0.08	0
Yellow	433030	KCO_3	0.44	+0.04	+0.04	0
Green	429628	KCO_3	0.44	+0.02	+0.02	0
Violet	433243	NaCl	0.76	+0.06	+0.05	0.01
		Li_2SO_4	0.85	+0.12	+0.12	0
Violet	606052	Li_2SO_4	0.85	+0.02	−0.03	0.05
		NaCl	0.76	+0.02	−0.05	0.07
Gray	541550	KNO_3	0.94	−0.02	−0.01	0.01
Gray	613713	KNO_3	0.94	+0.09	+0.07	0.02
Gray	613741	KNO_3	0.94	+0.07	+0.03	0.04
Gray	613732	K_2SO_4	0.97	+0.12	+0.01	0.11

[a] Average values determined from references shown in Table 3.

(RH) ($a_w \times 100$) may be taken over a range of 15–95% RH (0.15–0.95 a_w).

3. a_w values were checked until successive readings made 5 min apart gave a change of less than 0.005 a_w. For salt solutions, this took about 30 min; for the foods about 1 hr.

4. When not in use, the sensor was stored at ambient a_w (approx 0.25) or over saturated $MgCl_2$ solution.

The thumbwheel calibration was checked against standard saturated salt slurries and was found to be linear between a_w 0.35 and 0.93.

The Relative Humidity Indicator, Model 400D (General Eastern Corp., Watertown, Mass.) is another type of electric hygrometer and was tested at M.I.T. The sensor is sensitive to changes in resistivity of a sulfonated polystyrene matrix as a function of the water molecules present (General Eastern Corp., Bulletin 400-0174). This model gives a digital readout of % RH. For this study, a proportional signal was recorded simultaneously by a Heath IR-18M strip chart recorder. Since the instrument was not correctly calibrated as it came from the manufacturer, a new standard curve was generated using saturated salt solutions and was used to correct the readings. Determinations were done in the adsorption mode.

The procedure for the humidity indicator was as follows:

1. The sensor was sealed over the sample in the 4 oz snap cap vial.
2. A reading was taken from the digital display when the recorder indicated equilibration of the sensor in the air space over the sample.
3. The reading was corrected by using the calibration curve to obtain the experimentally determined a_w.

The hygrometer (Hygrodynamics, Silver Springs, MD) study done at the Univ. of Minn. required the use of several sensors since they are accurate only over particular a_w ranges for which the manufacturer supplies a graph of reading value vs % relative humidities. Since the sensors used were of varied and uncertain age and history, recalibration of each sensor was necessary so that a correction factor could be applied to the graph. The correction factor should be the same regardless of the salt used in calibration as long as it was within that sensor's range of sensitivity. This correction factor should also be applicable and accurate over the full range.

Most investigators have used variable periods of equilibration time before reading time such as 20–30 min (Fett, 1973); 8 hr (Plitman et al., 1973); and 24 hr (Vos and Labuza, 1974). The uncertainty in length of time stems from the fact that Hygrodynamics does not give a recommended time for analysis. The manual does state that a sensor will reach 65% of the equilibrium value in 3 min (Bulletin #SB-20). A time course on the equilibration of two of the sensors used in this study showed that after 24 hr the sensor had reached a maximum value, thus 24 hr was used for all samples which should be more than adequate for all sensors or sample types.

The procedure for the Hygrometer was as follows:

1. Samples with a wide range in expected a_w were chosen for 1 day's analysis. The samples were put into 4 oz screw-cap jars to half-full, but not touching the probe which was in the jar lid. The appropriate sensors were sealed onto the jars. Parafilm was stretched around the sensor end of the jar to ensure a moisture barrier.
2. After 24 hr ± 0.5 hr, the sensor was connected to the instrument and a reading was taken from the Hygrodynamics meter.
3. The sensor was then removed and placed in a vacuum desiccator over drierite and a vacuum was pulled. The desorption process got rid of water as well as other volatiles like glycerol and propylene glycol, which could adsorb on it.
4. After 24 hr the sensor was taken from the desiccator and sealed over the next sample to be analyzed.
5. All determinations, samples and standards were done in duplicate. After a correction factor was determined for a sensor, it was used in analysis of unknown samples.
6. When all samples for a particular sensor had been analyzed (including the 24 hr desorption process), each sensor was recalibrated over the same saturated salt slurry that was used to establish the correction factor. This was done by sealing the "dry" sensor over the slurry for 24 hr and then taking a reading as described above.

It was found that some sensors gave different correction factors when retested after 3 or 4 samples during the 3-month period of the study, as seen in Table 4. The sensors for the lower a_w range gave reproducible correction factors. The red, yellow and green sensors maintained the same correction factor throughout the course of the study. These sensors were not tested against a second salt. Most sensors for the higher a_w range changed their correction factor during the course of the study from 0.01 to 0.11 a_w units. In addition, one of the violet sensors (#433243) required a different correction factor depending on the saturated salt slurry used for calibration. With NaCl the factor was +0.05 to +0.06, but with the Li_2SO_4 it was +0.12. This is unexpected and undesirable and suggests that the calibration curves and correction factors are not linear. When this problem was discovered, the calibration factor applied was the one established nearest to the *time* of analysis of each particular sample. This could not be based on a value near the a_w of the sample, since that was unknown until after a correction factor was applied.

The samples to be tested by the Sina-scope (Sina Ltd., Zurich, Switzerland, marketed in the U.S. by Beckman) were performed by W. McCall at Armour Food Co., Oakbrook, Ill. The a_w was determined on a Sina-scope equipped with an indicator/recorder which indicated when equilibrium had occurred between food, air space and sensor element. Since the sensing system and sample space are in close contact, equilibration is rapid and occurred within 30 min to 1 hr. The system calibration was expanded to measure a_w over the range of 0.76–1.0. Other ranges are available, as well as one to cover from 0.05–1.0. For this study the dry soup mix was out of the range used thereby making accurate reading impossible. The Sina-scope sensor was equipped with a mechanical filter to protect it from dust, oil and water vapor condensation. A chemical filter can be used to protect the sensor from chlorine, formaldehyde, ammonia, sulfur dioxide, hydrogen sulphide, amino-acids, hydrocarbons and oil droplets. Thus, volatile chemicals in the samples should not cause interference from glycols in the cat food or Hennican. This is a definite advantage over the Hygrometer sensors. This filter was unavailable during this study, however, the necessity for such a filter may be realized by the results found.

The procedure for the Sina-scope was as follows:

1. The sample was put into a plastic sina dish after room temperature equilibration. The open dish was put into the base and the Sina-scope sensor was screwed down forming an air-tight seal.
2. The manufacturer furnishes check tablets which are combined with a few drops of water to check the calibration of the instrument.

The tablets are salts and thus saturated solutions are supposedly formed. However, using their procedure, it was obvious that too much dry salt was exposed in the air space which can result in an incorrect calibration. In spite of this, the manufacturer's calibration procedure was used.

3. The indicator/recorder was switched on and time was allowed for the recorder chart to indicate equilibration. The % relative humidity (RH) is then read directly from a bar scale and corrected with the calibration.

The handbook for the Sina-scope references Wexler and Hasegawa (1954) for the a_w of the salts suggested for calibrating the sensor. Unfortunately, mistakes were made in referencing this work. The handbook quotes the values to three decimal places despite the author's qualification of the values for salts. Wexler and Hasegawa used dew-point measurement to determine the a_w resulting from various salts at various temperatures. They then plotted data for the salts from 20 different references on the graphs of the curves they obtained for a_w vs temperature. It showed the scattering of data for a salt by different authors using different techniques; the standard deviation in most cases being ±0.03 a_w units and with one salt, magnesium nitrate, ±0.06 a_w. Thus, again an average value may not be the true value.

The isopiestic method of a_w determination was modified from that described by Fett (1973) in which sodium caseinate was the adsorption substrate. The procedure described by Vos and Labuza (1974) was followed using microcrystalline cellulose as the adsorption substrate. The cellulose is a more stable and thus superior substrate. This hybridized method is called the Fett-Vos isopiestic method of a_w determination and was done at the University of Minnesota.

The procedure for the isopiestic or equilibrium moisture adsorption method was as follows:

1. Approximately 100g of the sample was placed in the bottom of a 215 cm plastic desiccator (vacuum type).
2. Duplicate samples of 1.6g (to 0.0001g) of predried microcrystalline cellulose (Avicel, PH-101) were weighed into 35 ml glass weighing dishes with lids. The dishes (without lids) were then placed on the porcelain plates in the desiccators over the food.
3. The desiccators were closed, evacuated for about 1 min and then held at 22°C for 24 hr.
4. After 24 hr, the vacuum on the desiccators was very slowly released. Rapid air current within the desiccator will result in a loss of cellulose from the weighing dishes and failure of that determination. If the desiccator has not maintained a good vacuum during the 24 hr isopiestic procedure, the data from the sample within will be erroneous.
5. The dish (with the lid replaced) and the cellulose were re-weighed and the change in weight recorded. The moisture content (g H_2O/g dry cellulose) was calculated and the a_w read off the standard cellulose isotherm which was prepared previously using sulfuric acid water mixtures as the medium. These solutions were measured on the VPM and agreed with standard values.

This method of a_w determination is not recommended for samples that are subject to foaming, such as protein solutions. The protein samples (HPH and NZ-amine and H_2O) foamed excessively during the evacuation of the desiccators. A slight modification in procedure was used for these two samples. The samples were degassed prior to evacuation. The degassing was done by putting the sample in a desiccator and evacuating for 3 min interrupting periodically to prevent sucking the sample out through the vacuum hose. When degassed, the sample boiled but the profuse foaming was eliminated. During the procedure 0.5g of water/100g sample was lost; probably not enough to alter the a_w since the moisture content is greater than 55g/100g sample. A change of 5.0g is needed to change the a_w by 0.01.

Microbial growth during the 24 hr equilibration must be prevented. This is possible by the addition of antimicrobial agents (K-sorbate) and by using aseptic technique.

The range of accuracy of this method is limited by the slope of the cellulose isotherm. The best accuracy is found in the high a_w range, between a_w 0.81 and 0.96, the IMF range and slightly above. This procedure is highly applicable for heterogeneous samples due to the large sample size used. Application of the Fett-Vos method for samples containing volatiles such as propylene glycol or 1,3-butylene glycol should be exercised cautiously. Adsorption of vapors other than water vapor onto the cellulose will provide erroneous results.

The vapor pressure manometric method (VPM) is one of the best methods for a_w determination as it gives a direct measure of the vapor pressure exerted by the sample. Devices based on this method have been described by Taylor (1961) and Labuza (1974). The a_w is calculated from the ratio of the vapor pressure of the sample to that of pure water at the same temperature. This method was performed at the University of Minnesota.

The procedure for the VPM method was as follows:

1. A 10–30g sample was put into a special 50g sample flask (with 24/40 top) and sealed onto the apparatus.
2. The airspace in the apparatus is evacuated (via vacuum pump) to less than 200 microns (Sample is excluded).
3. The space in the sample flask was then evacuated for 30 sec to 2 min depending on the sample. This time should be kept to a minimum to prevent loss of H_2O from saturated solutions; however, this is not of great significance when following the above procedure as long as it does not actually dry out. Then the stopcock across the manometer is closed.
4. The level of oil in the manometer (Apeizon B oil) will respond to the vapor pressure exerted by the sample. The system was equilibrated at a constant temperature until the manometer oil showed no change in height. This usually took 40–60 min.
5. The difference in height of the legs of the manometer was recorded as ΔH_1 at the temperature of equilibration. The sample should be water jacketed. Two thermometers, one in the water jacket and one in the air hung from the VPM frame, were used for temperature equilibration. Temperature equilibrium exists when these two precalibrated thermometers showed the same temperature for at least 10 min.
6. The stopcock over the sample was closed and the one over the desiccant was opened. The moisture in the air space of the system absorbs onto the desiccant. As this occurs, the manometer oil lowers. After no further change in the manometer leg occurs, usually 10–20 min, the difference in the legs was recorded as ΔH_2. This difference was due to gases and volatiles lost from the sample and air that leaked into the system.
7. The pressure exerted by the sample water vapor, $\Delta H_1 - \Delta H_2$ (cm), is divided by the pressure that pure H_2O would exert at the same temperature to give the water activity.
8. The drierite stopcock was then opened and the drierite and flask are evacuated. The vacuum was broken on the sample by passing air into the flask through a 3-way stopcock. The sample is taken down from the apparatus.
9. The airspace throughout the system was again evacuated for 15 min. It was then ready for the next sample.

Temperature control is one of the most critical factors involved in accurate determination of a_w by the VPM. Sample and air measurement by thermocouples connected to a multipoint recorder should be used to ensure equivalence of temperature. Volatiles other than water may contribute to the pressure exerted by the food, giving erroneous results. Considerable work on this question concerning humectants like glycerol and propylene glycol has shown that these glycols have no significant effect on the a_w determination.

It is impossible to perform a_w determination on samples containing high numbers of bacteria or mold. Their respiration prevents vapor pressure equilibrium. The oil in the manometer changes constantly with time. This subject has been discussed previously with regard to saturated salt solutions. The same problem was experienced with a sample of uncooked Thuringer. After cooking, the bacteria were inactivated and a reasonable a_w was obtained. Another disadvantage (or uncertainty) of a_w determinations by the VPM is that some moisture is lost during the evacuation step of the procedure. This could be critical due to the small sample size. The actual loss for each type of sample may vary depending on the functional properties of the sample, e.g., texture, viscosity and porosity. Sood and Heldman (1974) have shown this to be insignificant for foods of low moisture (a_w 0–0.6). In one of our tests, a sample of 30g of high moisture cheese (40%) was evacuated for ½ min and 2 min and weighed after each time period.

The results showed that in 30 sec, less than 0.05g of water was lost from 30g of cheese, while in 2 min only 0.14g was lost. Thus, about 1% of the water was lost from the cheese. Since cheese is at a high a_w with a steep isotherm, the effect on a_w should be very small. From this it was felt that the normal evacuation of a sample (30 sec to 1 min) will not significantly alter the a_w of high a_w samples since most isotherms are steep above 0.60.

A test of measurement precision was performed using the devices at the University of Minnesota. Five determinations for Li_2SO_4 were done by the Hygrometer using a single sensor calibrated over NaCl, the Fett-Vos and the VPM methods. The temperature during the analysis was 22°C ± 1°C. The Li_2SO_4 was made up in one batch of slurry by adding distilled-deionized water as usual. All glass utensils were used in its preparation. Dirt and metal ions can affect the vapor pressure of salt slurries. Samples of this slurry were taken for analysis by the three methods. The procedure for each method has been discussed before.

RESULTS & DISCUSSION

AS SEEN IN TABLE 5, the VPM gave the best precision in the limited study on Li_2SO_4. However, the VPM value was low when compared to the standard values listed in Tables 2 and 3. The Fett-Vos procedure also gave excellent precision, but reads high by 0.03 units. The Hygrometer readings were low, and had a standard deviation of ±0.04. The values for Li_2SO_4 ranged from 0.80–0.91. Based on these results, one should not rely on the standard value reported but one could use the VPM or Fett-Vos method with a correction factor for an accepted value. Why the values differ from the literature is not known; however, it is believed that the literature values may not be correct.

The results of the collaborative study are presented in Table 6 for the values obtained for the standard saturated salt slurries and in Table 7 for the foods and food systems. The average value for each duplicate determination is listed. It is suggested that an average of duplicate determinations always be made. As seen in Table 6, the VPM gave results that were closest to the standard values listed in Table 3. The average difference from theoretical was 0.01 a_w unit. The electric hygrometers, Humidity Indicator 400D, Humichek, Hygrometer and the Sina-scope all had an average deviation from theoretical of 0.02. The range of the particular Sina-scope used was limited to $a_w > 0.81$ and the Humichek to a_w below 0.95. The Brady Array had the largest deviation from the standards. The Fett-Vos method had, for only a small number of salts tested, an average deviation of 0.02. As noted, use of this method is limited to the higher a_w's.

Table 5–Test of precision on Li_2SO_4 at 22°C

Trial	VPM 22°C a_w value	Hygrometer 22°C a_w value	Fett-Vos 22°C a_w value
1	0.82	0.88	0.88
2	0.82	0.83	0.88
3	0.82	0.81	0.88
4	0.82	0.78	0.88
5	0.83	0.85	0.88
Absolute average	0.83 ± 0.01	0.83 ± 0.03	0.88 ± 0
1[b]	0.83	0.87	0.87
2[b]	0.83	0.84	0.87
3[b]	0.84	0.91	0.85
4[b]	0.83	0.80	
5[b]		0.80	
Overall average[a]	0.83 ± 0.01	0.84 ± 0.04	0.87 ± 0.01

[a] Average of all results = 0.845; Rockland value = 0.85
[b] Previous studies over 2-yr period in lab

The relative accuracy of these methods was based on comparison to literature values. There is an intrinsic error in this as the absolute a_w's of the slurries may not be what is listed as "theoretical." The reasons for this are:

1. The standard value was found by averaging values from the literature. Different values are reported for a particular salt and temperature.
2. The values in the literature were determined by different methods which have different accuracies. For instance, Wexler and Hasegawa (1954) used a dewpoint apparatus (±0.03) while Rockland (1960) used an electric Hygrometer (±0.015, Hygrodynamics Bulletin, #SB-20). The accuracy of the latter may in fact be poorer than reported.
3. The literature is cross referenced, giving values unequal weight depending on how often a particular author is cited.
4. The a_w's of the slurries are temperature dependent. Some are greatly influenced by changes in temperature and, as a rule, such changes should be avoided. For instance, $Zn(NO_3)_2$ slurry has an a_w of 0.38 at 20°C and 0.31 at 25°C.
5. Investigators handle the literature values in different ways. If analyses was performed at 22°C, values at 20° and/or 25° have been averaged (depending on what is available), or values at only the temperature closest to that of analysis have been averaged, i.e., 20°C. Thus although one can state that the VPM is best, this study points out that perhaps some National Bureau of Standards sample should be made and all investigators should use a correction factor with their method for reporting a_w. In addition, Table 5 points out the possible need for multiple determination with some of the methods.

A study made by the U.S. National Bureau of Standards comparing published results of various investigators found that % relative humidity (a_w × 100) values for salt slurries in the literature fell within a range of ±1.5% RH (±0.015 a_w) from the NBS data (Wexler and Hasegawa, 1954). Thus, to date only a qualified expression of a_w should be made. Using modern technology, an absolute a_w of saturated salt solutions should be established. Since most methods rely on the salts for calibration, a direct measurement of the vapor pressure of the saturated salt slurries would give the best results. The method

Table 6–A_w results for saturated salt slurries[a]

Material	Theoretical a_w	Direct VPM	Electric Hygrosensors: Humidity indicator	Humichek (Beckman)	Hygrometer (Hygrodynamics)	Sina-scope	Resonance Brady Array	Isopiestic Fett-Vos
$MgCl_2$	0.33	0.32	NT	0.31	0.32	BR	0.31	BR
$Mg(NO_3)_2$	0.54	0.53	0.52*	0.53	0.46	BR	0.50	BR
$(NH_4)_2SO_4$	0.81	0.80	NT	0.80	0.79	0.78	0.80	0.84
$CdCl_2$	0.82	0.82	0.86*	0.81	0.83	0.83	0.79	0.83
Li_2SO_4	0.85	0.84	0.86*	0.84	0.83	0.83	0.86	0.87
Na_2HPO_4	0.96	0.96	NT	0.93	0.96	0.95	0.89	BR
K_2SO_4	0.97	0.97	0.96*	BR	0.97	0.99	0.98	BR
Avg difference from theoretical a_w		0.01	0.02	0.02	0.02	0.02	0.03	0.02

[a] NT = not tested; * = based on a single determination; BR = beyond range of the method used

Table 7—A_w results for foods and food systems[a]

Material	Theoretical a_w	Direct VPM	Electric Hygrosensors				Resonance Brady Array	Isopiestic Fett-Vos
			Humidity indicator	Humichek (Beckman)	Hygrometer Hygrodynamics	Sina-scope		
Dry soup mix		0.21	0.21*	0.26	0.23	BR	0.23	BR
Thuringer		0.95	NT	NT	0.93	0.95	NT	0.94
Cheese		0.97	0.92*	0.93	0.94	0.94	0.89	0.95
Bread		0.97	0.94*	0.93	0.95	0.95	0.92	0.95
IM Pet food		0.87	NT	NT	0.90	0.86	NT	0.90
Hennican	0.91	0.90	0.90*	NT	0.90	0.88	NT	0.90
Hennican + 2% glycerol		0.87	0.90*	0.88	0.86	0.88	0.89	0.89
Hennican + 2% propylene glycol + 2% butylene glycol		0.86	0.88*	0.87	0.87	0.87	0.90	0.89
Semolina + egg		0.93	0.92*	0.93	0.89	0.92	0.89	0.90
Protein medium	0.90[b]							
a.		0.88	NT	NT	0.91	0.90	NT	0.91
b.		(i)	0.90	0.89	0.92	0.90	0.87	(i)
Protein medium	0.93[b]							
a.		0.90	NT	NT	0.93	0.92	NT	0.91
b.		(i)	0.92*	0.92	0.93	0.92	0.91	(i)

[a] NT = not tested; BR = beyond range; * = based on single determination; (i) = impossible due to foaming.
[b] Troller (1971)

of choice is the VPM, since it functions independently of calibration by saturated salt solutions and measures a static physical phenomenon.

The results for the foods are shown in Table 7. The data for high a_w samples are quite variable. The a_w of cheese was determined to be from 0.89 (Brady Array) up to 0.97 (VPM). The Hygrometer and the Sina-scope gave a value of 0.94. Bread also gave a wide range of values. Again the higher VPM value may be due to the problem of accurate temperature control and monitoring in the high a_w range (Labuza, 1974). The Hygrometer, Sina-scope and Fett-Vos method all gave a value of 0.95. This trend does not exist for all samples; however, all other samples have a_w's determined within a range of ±0.02. Thuringer was measured as a_w 0.95 (VPM and Sina-scope) and as a_w 0.94 and 0.93, respectively, by the Fett-Vos method and the Hygrometer. The data for the IMF samples are closer than for the high moisture samples.

Some of these samples have humectants (glycols) added, but the levels are so small that there should not be any significant interference. The amount of the most volatile glycol (1,3-butylene glycol) in a VPM sample (2% of 30g) does not contribute significantly to the measured a_w by VPM. Accumulation of glycols onto sensors can cause erroneous readings but the desorption process used for all sensors in this study excluded that source of error. Adsorption of glycols onto the cellulose used in the Fett-Vos procedure could also be a source of error if the glycol was a significant proportion of the composition. No such samples were tested in this study. The glycols were present as only 2% of the composition. The a_w values of the Hennican plus 2% glycerol were from 0.86 (Hygrometer) to 0.90 (Brady Array), the same extreme values as the Hennican with less humectant.

The bacteriological media, NZ-amine and PHP, was made up and analyzed on two occasions to compare reproducibility of preparation. Both trials were analyzed by the Hygrometer and Sina-scope. Excessive foaming during evacuation made the analysis difficult to impossible for the VPM and the Fett-Vos method. Only the first trial was successfully analyzed by those two methods. The three instruments tested at M.I.T. were used only in the second trial. There is good agreement between Trials 1 and 2 by the VPM. The a_w of each system, however, varies depending on the method of analysis. The 0.90 media was measured as 0.90 (VPM) and as 0.93 (Hygrometer). In this case, the loss of moisture from the samples for VPM analysis could be significant. Extensive degassing (3 min) of the media was required to produce a sample that could undergo the VPM evacuation step. The VPM values, therefore, may be low for these two systems.

CONCLUSIONS

IN GENERAL, the data for the foods varied by ±0.02 a_w units depending upon the method of determination used, as was the case for the salt solutions. The foods with a higher a_w gave a wider range since the VPM data were always higher. However, no method seemed consistently high or low or better by these comparisons. As seen, however, one would question the validity of literature results either reported to three places for a_w or of absolute values for lower a_w limits of microbiological growth.

Based on this study, some recommendations can be made for a_w determination.

1. Equilibration at the temperature of analysis is a must; 24 hr is sufficient for most samples, but more time may be needed for heterogenous samples.
2. Moisture transfer during equilibration or analysis must be kept at a minimum, whether from the atmosphere, sample-jar head space, or from the frozen state.
3. Sample history must be known. Extremes in temperature must be avoided if not a part of a process parameter. This treatment may affect the water in the sample from an adsorption or desorption situation. Change in temperature can cause a cross-over from one branch of a hysteresis loop to the other, resulting in a change in a_w.
4. Sensors, when used, should be maintained under optimum (dry) conditions and calibrated on a regular basis. Equilibration of sensor with sample should be complete.
5. Standard salt slurries must be pure. Those at $a_w > 0.95$ must prove free of microbial activity.

6. Constant temperature must be maintained during calibration of sensors. Slight temperature fluctuations may cause the vapor in high a_w salts to condense. The resulting a_w may then be between that of the slurry and that of water, 1.0. Other details for use of saturated salt slurries appear in a publication put out by Hygrodynamics (Bulletin #5).

7. Contamination of sensors must be avoided.

Once standard values for salt slurries are established, they may be used to set and maintain calibration of the variety of a_w or relative humidity measuring methods. Any of the methods tested could be used in determining a_w. A few are less expensive, faster and/or more accurate than others. Positive and negative aspects must be weighed when choosing a method for basic research or quality control use.

If regulations are made governing a_w limitations for a particular product, then processors should standardize the methodology used for that particular product. Only then can comparisons be made with accuracy of ±0.01 between laboratories or processors. It would be good for the National Bureau of Standards to prepare these standards.

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Ms received 9/16/75; revised 1/15/76; accepted 1/20/76.

This is paper No. 9219 from the Univ. of Minnesota Experiment Station. This study was supported in part by Univ. of Minn. Expt. Stn. Project No. 18-72 and by NASA, Contract #NAS-9-12560, Lyndon Johnson Space Center, Houston, Texas.

Presented at the 35th Annual Meeting of the Institute of Food Technologists, Chicago, IL, June 8—12, 1975.

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Staphylococcus aureus CHALLENGE STUDY IN AN INTERMEDIATE MOISTURE FOOD

ABSTRACT

The effectiveness of several microbial inhibitors in preventing the growth of *Staphylococcus aureus* in an intermediate moisture food was tested. *S. aureus* was inoculated into the food at a_w's of 0.86–0.90 and pH 5.2 and 5.6. Not all inhibitors prevented growth. The effectiveness against *S. aureus* was a function of both pH and a_w. Of the compounds tested, methyl paraben, sodium benzoate, potassium sorbate, and calcium propionate proved to be the most effective against *S. aureus* at low concentrations.

INTRODUCTION

INTERMEDIATE moisture foods (IMF) have received widespread attention in recent years. The popularity of convenience foods, which can be stored at room temperature and which require no further preparation before consumption, has caused many manufacturers to develop intermediate moisture formulations for human and pet foods (Bone et al., 1974). The relatively low moisture contents which characterize IMF can result in the production of foods which are microbiologically shelf stable under conditions of room temperature storage. As the moisture content or water activity (a_w) of a food is lowered, the amount of water available for supporting microbial growth is lessened (Troller, 1973; Scott, 1957). Generally, yeasts and molds are able to grow in environments with lower a_w's than are bacteria. The pathogen, *Staphylococcus aureus*, is unusual in that it can grow down to a_w 0.83–0.84 and produce toxin down to a_w 0.86 as reviewed by Troller (1973) and Tatini (1973).

Previous work in our laboratories tested the effectiveness of several common food additives at a_w 0.85 in preventing the growth of the mold, *Aspergillus niger*, in a model IMF (Acott and Labuza, 1975). This a_w permits the growth of many molds but inhibits the growth of almost all bacteria, especially the growth of many potentially pathogenic bacteria. However, in the formulation of IMF, it is often desirable to have an a_w of somewhat greater than 0.85 to create an acceptable product from a textural standpoint. As stated, the growth of *Staphylococcus aureus* can begin at an a_w of about 0.83–0.84 depending on the product (Tatini, 1973). During growth, *S. aureus* produces enterotoxins which can cause food intoxication and thus pose a potential health hazard if the a_w is at 0.86 or above.

As reviewed by Minor and Marth (1972), staphylococcal contamination is very common in a wide variety of foods. If an IMF ingredient became contaminated with *S. aureus*, enterotoxin production might occur prior to final processing if there were considerable *S. aureus* growth. Thus the fact that the a_w was less than 0.86 would not guarantee the food to be safe. The release of enterotoxins by *S. aureus* can occur if populations exceed about 10^7/CFU/gram (Tatini et al., 1971; 1973). However, if conditions are correct, enterotoxin may be produced by high populations of nongrowing cells (Markus and Silverman, 1968, 1969).

The present study was designed to determine the effects of microbial inhibitors which were previously tested in IMF products on the growth of *S. aureus* F265. Enterotoxin production was not measured in this study. The model IMF was formulated to the a_w range 0.86–0.90 which would support the growth of staphylococci, thus eliminating the effect of an inhibitory water activity.

The pH of a food system also may influence the effectiveness of a microbial inhibitor. The acid type inhibitors must be in the undissociated form to be inhibitory to microbes (Sauer, 1972; Ingram et al., 1956). As the pH of the food to which the inhibitor has been added is lowered, the proportion of the inhibitor in the undissociated state increases and increases the effectiveness. Chichester and Tanner (1968) suggest that this is because the undissociated form is highly lipid soluble and accumulates in the lipid structures of the cells inhibiting cell metabolism.

Parabens and polyhydric alcohols were also studied. The parabens which are also an acid type inhibitor are very effective against many microbes, especially mold and Gram positive bacteria (Furia, 1968). The parabens are more effective at a high pH than are the other acid-type inhibitors. Methyl and propyl paraben were used together in one test system because the best inhibitory effect is often obtained by using a combination of the two compounds (Chichester and Tanner, 1968). The polyhydric alcohols bind water and inhibit microbial growth by lowering the a_w of the food system. These compounds may inhibit microbial growth in other ways in addition to lowering the a_w.

Caproic acid, a long-chain fatty acid, has been patented for use instead of potassium sorbate in intermediate moisture dog foods (Haas, 1973). This compound has not been approved by the FDA for use in foods, but was also tested.

MATERIALS & METHODS

Model IMF formulation

The composition of the model IMF (Hennican) is shown in Table 1. The natural pH of this system was 5.6. The unsalted, hulled peanuts (Skippy Co., Minneapolis, MN) were ground in a blender. The chicken (Aslesen's Banquet Table, canned deluxe boned) was freeze dried and ground in a silent cutter (Hobart Co., Model 84181). Nonfat dry milk, ground peanuts and chicken were mixed in a 500 ml Brabender-Farinograph bowl.

Raisins were ground in a food grinder and blanched in a microwave oven for 1.5 min to destroy active enzymes. The raisins, peanut butter (Skippy, creamy style), and honey were added to the dry ingredients in the Brabender-Farinograph bowl as mixing continued. The water was added slowly and the IMF was mixed at high speed for 5 min.

Initial work showed that the Hennican contained large amounts of a natural mold contaminant. The growth of this mold inhibited staphylococcal growth in the model IMF. Pimaricin, an antimycotic, has been found to be effective in inhibiting over 500 fungi but has no effect on

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the growth of bacteria or viruses (Clark et al., 1964). As shown in Figure 1, in the system to which no pimaricin was added, considerable mold growth occurred after 7–10 days and staphylococcal growth was inhibited; however, growth of the *S. aureus* F265 continued in the system to which pimaricin (0.002%) had been added and no mold was observed. Thus pimaricin inhibited mold growth in Hennican without inhibiting staphylococcal growth.

Addition of inhibitory compounds

The first part of the study was done by adding the inhibitory compounds to the model system at pH 5.6. In the second part, the system was acidified to pH 5.2 using citric acid so the effect of slightly lowered pH on the staphylococci could be measured. The pH was measured directly with a Beckman nonaqueous electrode. A pH of 5.2 was chosen because preliminary work showed that staphylococcal growth was best in our system at or above this pH, and at lower pH the food was organoleptically unacceptable. In a study by Scheusner and Harmon (1973), it was shown that no enterotoxin was detectable in foods below pH 5.0; however, as demonstrated by Tatini (1973) growth and enterotoxin production can occur at pH 4.0–4.5 depending on the medium. Thus, a pH of 5.2 should not prevent enterotoxin production by growing staphylococci. Since it was believed that a lowered pH would result in increased bacterial inhibition, lower levels of the inhibitors than those which proved to be effective at pH 5.6 were tested in the systems at pH 5.2.

The inhibitors tested in this study are shown in Table 2. The acid-type inhibitors were added at the desired levels to 50g of Hennican. It was found that these additives did not affect the a_w of the product. The polyhydric alcohols were added to 150g portions of Hennican in the desired amounts. The amount of each humectant required to lower the food to a_w's 0.86 and 0.88 had been determined previously experimentally. Unfortunately, the heterogeneity of the Hennican system made it very difficult to obtain exactly the desired a_w repeatedly with addition of the humectants. From each 150g system, 100g were used to measure the a_w and 50g were used for inoculation with *S. aureus*. The Fett-Vos isopiestic method for a_w determination was used in this study (Vos and Labuza, 1974). The addition of the inhibitors did not markedly affect the pH of the Hennican systems.

Inoculation with *S. aureus* F265

Each 50-g portion was inoculated with a *Staphylococcus aureus* F265 culture which had been grown in trypticase soy yeast extract broth at 23°C for 24 hr. The culture was diluted to obtain the desired population for inoculation of the test systems. The level of inoculation was approximately 3×10^5 colony forming units per g of food. After thorough mixing, each system was divided into ten 5-g samples which were placed in 60 × 15 mm plastic petri dishes. The samples were stored in desiccators over saturated salt solutions.

Table 1–Hennican formulation

Ground peanuts	13.37g
Ground freeze-dried chicken	13.37g
Non-fat, dry milk	9.73g
Ground raisins	26.73g
Peanut butter	3.53g
Honey	1.44g
Water	33.00g
Pimaricin	0.002g
A_w = 0.91 pH = 5.6	

Conditions of storage

Storage within a desiccator over the appropriate saturated salt solution maintains the proper a_w of the samples by preventing them from drying out. The saturated salt solutions used in the desiccators for storage were: $BaCl_2$ (a_w = 0.90), $ZnSO_4$ (0.88), and KCl (0.86). Samples to which no inhibitors (controls) and acid-type inhibitors had been added were stored at a_w 0.90. Samples to which low levels of polyhydric alcohols were added were stored at 0.88 and those with higher levels of these compounds were stored at 0.86, as shown in Table 2. Samples were stored at 22°C.

Enumeration of the *S. aureus* F265

Five-g samples were combined with 45g of sterile phosphate buffer and blended for surface plating on trypticase soy agar to which 5g of yeast extract per liter had been added. The plates were incubated at 37°C and colonies were counted after 48 hr. The samples were plated

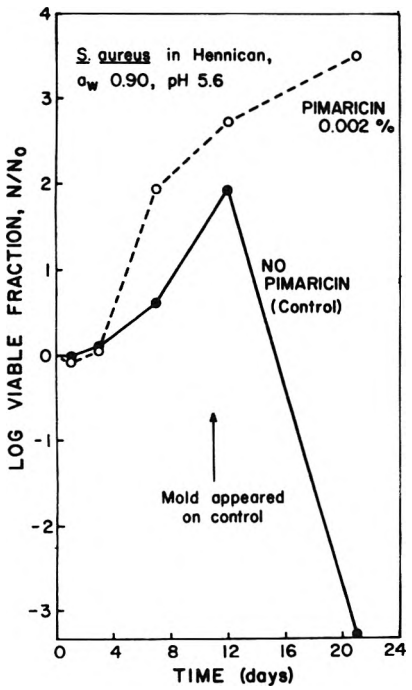

Fig. 1–Effect of pimaricin on S. aureus and mold growth in an intermediate moisture food.

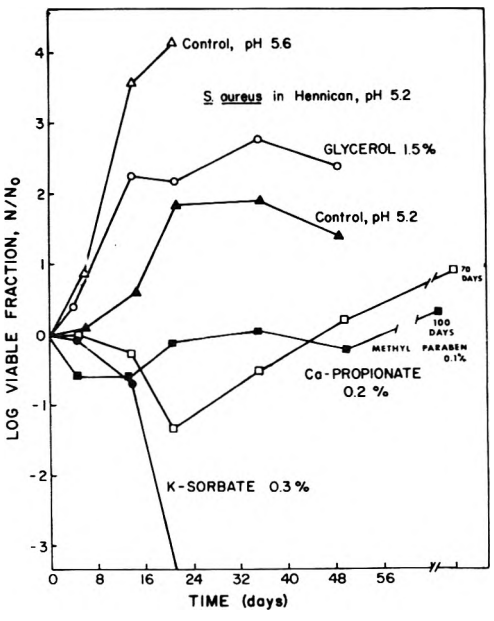

Fig. 2–Effectiveness of various conditions and additives on S. aureus in an intermediate moisture food at a_w 0.9.

for enumeration of the *S. aureus* immediately after inoculation and at various time intervals to monitor the bacterial population changes.

RESULTS & DISCUSSION

IN MONITORING the staphylococcal population changes throughout the storage periods, several types of growth patterns were observed. Figure 2 shows a graph of population changes in some representative Hennican systems at pH 5.2.

As can be seen from Figure 2, there were some systems in which the staphylococcal populations increased rapidly during storage. In the nonacidified control at pH 5.6 *S. aureus* growth was rapid. The control system at pH 5.2 showed slower yet still noticeable growth. When glycerol (1.5%) was added to a pH 5.2 system, growth was more rapid than without its addition yet slower than in the pH 5.6 control system. Glycerol probably acted as an energy source for the staphylococci (Patsch and Hoehne, 1967).

In the methyl paraben (0.10%) system, a bacteriostatic response is illustrated. The population level changed very little during 100 days of storage. Since staphylococcal growth did not occur in this system, methyl paraben was assumed to be an effective inhibitor of staphylococcal growth.

In the potassium sorbate (0.30%) system, the bacterial population decreased continuously. This compound proved to be an effective inhibitor and inactivator of staphylococci at this level of addition.

Another growth trend is illustrated by the calcium propionate (0.20%) system. As shown, the population decreased within the first 3 wk of storage but then began to increase steadily. This delayed increase in staphylococcal growth may have been due to the eventual metabolic reduction of the inhibitory propionate which decreased the compound's inhibiting effects. If population monitoring had been terminated within the first few weeks of storage when the population levels appeared to be decreasing, the later increases would have been undetected. This illustrates the vital importance of extended storage periods in challenge studies. In our work, platings to monitor staphylococcal growth were continued in all cases until a definite growth pattern was established. The growth patterns shown in Figure 2 are from systems at pH 5.2 but similar types of growth trends were shown at pH 5.6.

Table 3 shows the levels of the compounds which proved to be effective for inhibiting staphylococcal growth in Hennican at the two pH's tested. As expected, decreasing the pH of the model system increased the effectiveness of several of the inhibitors. In the case of the strongly acid dependent inhibitors, decreasing the pH of the test system decreased the dissociation of these compounds, enhancing their inhibitory effects, as described previously. In other systems, it is likely that decreasing the pH caused increased stress for the staphylococci. *S. aureus* grows best near neutrality. Although there may not have been a synergistic effect between the increasing acidity and these inhibitory compounds, an additive effect between the acidity and the inhibitor resulted in greater microbial inhibition at the lower pH.

The polyhydric alcohols used to lower the a_w of the systems varied in their effectiveness. Propylene glycol and polyethylene glycol 600 were effective at high levels of addition but did not prove effective at lower levels even with the additional stress of lowered pH. Neither glycerol nor sorbitol proved inhibitory even at high levels. It seems likely that these compounds were metabolized by the staphylococci. When compared to the other polyhydric alcohols, 1,3-butylene glycol was found to be an effective inhibitor at the lowest level of addition. At the present time, this compound is not approved by the FDA for use in foods as a microbial inhibitor. The results of this study indicate that 1,3-butylene glycol appears to be an effective inhibitor of *S. aureus* F265 as was found by Frankenfeld et al. (1973) and Plitman et al. (1973). It should be investigated for possible approval by FDA.

Methyl paraben proved to be more effective than propyl paraben at the levels of addition employed. The combination of methyl and propyl parabens did not prove to be effective. It should be noted that methyl paraben was added at a higher level than was propyl paraben or the combination of the two parabens so this may explain the differences observed.

At pH 5.2, caproic acid was found to be a very effective inhibitor of *S. aureus* F265. This indicates that this compound is worthy of further study for use as a microbial inhibitor in IMF.

This study has shown that *S. aureus* F265 growth can occur

Table 2—Test systems for the inhibition study

Inhibitor	pH 5.6 % level (w/w)	pH 5.6 Storage A_w	pH 5.2 % level (w/w)	pH 5.2 Storage A_w
Methyl paraben	0.2[a]	0.90	0.07	0.90
	0.4[a]	0.90	0.10	0.90
Propyl paraben	0.03	0.90	0.03	0.90
	0.05	0.90	0.05	0.90
Methyl:propyl paraben 2:1	0.02	0.90	0.02	0.90
	0.05	0.90	0.05	0.90
Sodium benzoate	0.05	0.90	0.20	0.90
	0.10	0.90	0.30	0.90
Calcium propionate	0.20	0.90	0.05	0.90
	0.30	0.90	0.10	0.90
Potassium sorbate	0.10	0.90	0.10	0.90
	0.30	0.90	0.30	0.90
Caproic acid[a]	Not tested		0.10	0.90
			0.30	0.90
Propylene glycol	7.0	0.88	1.0	0.88
	10.8	0.86	3.0	0.88
1,3-Butylene glycol[a]	5.0	0.88	1.0	0.88
	10.0	0.86	3.0	0.88
Glycerol	1.5	0.88	1.5	0.88
	6.0	0.86	6.0	0.88
Polyethylene glycol 600	3.0	0.88	1.0	0.88
	10.0	0.86	3.0	0.88
Sorbitol	3.0	0.88	3.0	0.88
	10.0	0.86	8.0	0.88
Control	0	0.90	0	0.90

[a] Not approved by FDA or above FDA level

Table 3—Levels of compounds effective in preventing staphylococcal growth at high A_w

Inhibitor	% Level (w/w)	A_w
	pH 5.6	
Methyl paraben	0.4	0.90
Propylene glycol	7.0	0.88
1,3-Butylene glycol	5.0	0.88
Polyethylene glycol 600	10.0	0.88
	pH 5.2	
Methyl paraben	0.1	0.90
Sodium benzoate	0.1	0.90
Potassium sorbate	0.1	0.90
Calcium propionate	0.3	0.90
Caproic acid	0.1	0.90
1,3-Butylene glycol	3.0	0.88

at a_w's near 0.90 and thus pose a potential health hazard in intermediate moisture foods. The compounds tested in this study were found to be less effective in inhibiting *S. aureus* at a_w's ranging from 0.86–0.90 than they were in inhibiting *A. niger* at a_w 0.85. Lowering the pH of the model IMF system by the addition of citric acid increases the effectiveness of several of the inhibitors, most noticeably the strongly acid-dependent compounds which are most effective in the undissociated forms found in the greatest amounts at low pH values.

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Ms received 8/22/75; revised 12/9/75; accepted 12/16/75.

This project was supported in part by University of Minnesota Agricultural Experiment Station Project No. 18-52, and NASA Contract NAS 9-12560 from The Lyndon Johnson Space Center, Houston, Texas.

Scientific Journal Series Paper No. 9255, Agricultural Experiment Station, St. Paul, MN 55108.

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PREDICTING THE WATER ACTIVITY OF MULTICOMPONENT SYSTEMS FROM WATER SORPTION ISOTHERMS OF INDIVIDUAL COMPONENTS

ABSTRACT

Plots of log (water activity/mole fraction water) against the square of the mole fraction solute, usually give straight lines having slopes K. Norrish (J. Food Tech. 1966, 1: 25) used K derived for binary solutions in an equation for calculating water activities of multicomponent systems, but this equation gave inaccurate results when used on solutions where a deviation from linearity occurs, or when the linear portion of the plot does not go through the origin. By using both intercept and slope, and adding a correction factor that corresponds to the contribution of the intercept to the total solute interactions to Norrish's equation for multicomponent systems, an equation was derived that accurately predicted water activities of multicomponent systems. This concept of utilizing the intercept was applied to systems containing solids whose molecular weights are not well defined. Plots of log (water activity/moles water in 100g solution) against the square of the weight percent solids in the system, gave a series of straight line segments. Slopes and intercepts were different for each concentration range where the straight line segments lie. An equation was derived based on this equation for water activity as a function of concentration. In the derivation, moisture transfer between two components and equality of the water activities of all phases at equilibrium was considered. The derived equation predicted accurately equilibrium water activities of mixtures of two solids.

INTRODUCTION

THE DEPENDENCE of a number of deteriorative reactions in foods on the water activity is well established. In the food industry, routine water activity determinations are now employed in research and in quality control. The ability to predict the water activity of mixtures from the properties of the components would be useful in checking against erroneous determinations of water activity, and in formulating foods to the desired water activity.

A number of equations have been proposed for calculating the water activity of foods from the composition (Grover, 1947; Money and Born, 1951; Norrish, 1966). Raoult's law has been used by some authors to explain the dependence of water activity on the solute level and the moisture content of a food system (Kaplow, 1970; Bone, 1973; Ross, 1975). All of the equations that have been previously derived were tested on solutions of soluble solids. Ross' (1975) technique for calculating water activity from the composition appear to have some merit when used for soluble solids in water, but some problems remain when applying the technique in calculating the water activities of systems containing insoluble solids.

In this paper, equations were derived that could be applied in calculating water activities of systems containing insoluble solids.

EXPERIMENTAL

INITIALLY, water activities were determined using an electronic hygrometer (Hygrodynamics model 15-3001; Hygrodynamics Inc., Silver Springs, Md.). The reproducibility of the determinations using this instrument was not very satisfactory. All water activity values reported in this paper were obtained by measurement of the vapor pressure of water using a manometric technique.

The device is shown in Figure 1. The manometer was constructed at the University of Georgia glass blowing shop, and was similar to the device used by Taylor (1961).

All valves were V4 high vacuum valves. The system originally had the manometer directly joined to the sample flask with glass tubing, but difficulties were encountered because of frequent breakage of the glass when the sample flask was removed. The use of a short length of pure gum amber rubber tubing (sulfur free nitrometer tubing, 1/4 in. i.d. by 5/8 in. o.d., Fischer Scientific) between the manometer assembly and the ground glass joint that fits the center well of the three-necked sample flask, eliminated this problem. The rubber-to-glass seal with the 10 mm o.d. glass tubing was satisfactory when the overlap was at least 30 mm. In the system shown (Fig. 1), erroneous readings due to leaks were manifested by manometer readings that would not stabilize during the determination. Where leaks were encountered, this usually occurred when powdered material, liquids, or dust, were trapped on the surfaces of the ground glass joints. All determinations were done in a room maintained at 25°C. Anhydrous calcium chloride was used as a desiccant in the vapor trap prior to the vacuum pump, and "CENCO" Hyvac oil 93050 No. 3 (Central Scientific Company; Chicago, Ill.), with a specific gravity of 0.895 at 25°C, was used as the manometer fluid. A Precision Scientific (Chicago, Ill.) model 75 high vacuum pump, was used to evacuate the system.

The system was evacuated for a minimum of 10 min after which the stopcock between the two manometer legs was closed. With the other two stopcocks open, and the vacuum pump running, the system was allowed to equilibrate until two successive manometer readings taken 2 hr apart equalized.

The temperature of the sample in the three-necked flask used as a sample holder during the determinations, was monitored by a thermocouple introduced into the flask through a thermocouple well sealed off from the contents of the flask and constructed from a thin walled glass tube. Water was placed inside the thermocouple well to facilitate heat transfer between the thermocouple junction in the well and the sample

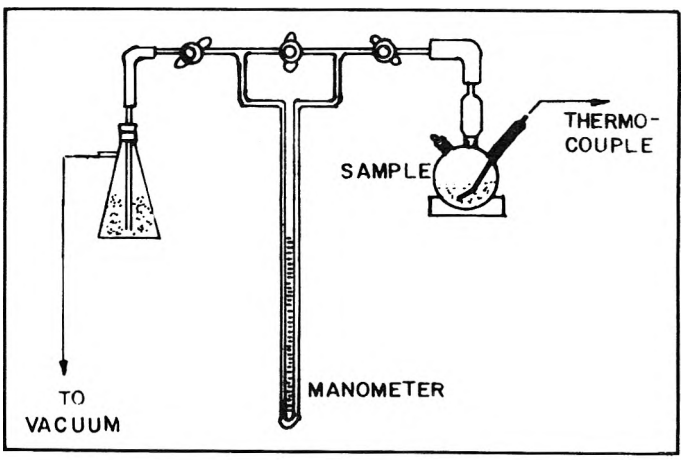

Fig. 1—Manometric system for determination of water activity.

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inside the flask. The temperature was recorded and was allowed to stabilize at a constant temperature for at least 30 min before the first reading of the vapor pressure was taken. Because of temperature cycling of the constant temperature room, sample temperature could be different (within 1°C) from room temperature at the time vapor pressure was read. The vapor pressure change however was very noticeable with a 0.1°C change in sample temperature, whereas a momentary change in room temperature by as much as 2°C did not affect the manometer reading as long as sample temperature remained constant. Measuring the sample temperature itself during vapor pressure manometric determinations of water activity improved the precision of the technique. Water activities were calculated from the ratio of the measured vapor pressure and the vapor pressure of pure water at the sample temperature.

A comparison of water activity determinations and literature values for solutions of sodium chloride and sucrose is shown in Table 1. The measured water activities were slightly lower by 0.01 water activity, when compared to the data reported by Robinson and Stokes (1959).

RESULTS & DISCUSSION

Norrish's equation applied to electrolyte solutions

Norrish's (1966) equation for water activity of binary sugar solutions is as follows:

$$\log \frac{a}{X_1} = K_{12} X_2^2 \qquad (1)$$

If $\log a/X_1$ is plotted against X_2^2, the slope is K_{12}. For multicomponent systems:

$$\log \frac{a}{X_1} = -[(-K_{12})^{1/2} X_2 + (-K_{13})^{1/2} X_3 + \ldots]^2 \qquad (2)$$

Where: X_1 = mole fraction of water; X_2; X_3 . . are mole fractions of components 2 and 3, respectively.

Constants K_{12} and K_{13} are slopes of plots of $\log a/X_1$ vs X_2^2 or X_3^2 for binary solutions of water and solute 2 and solute 3, respectively.

Figure 2 shows plots of Eq (1) for the system sucrose water, KCl-water, and NaCl-water, using the data reported by Robinson and Stokes (1959). The deviation of the electrolyte solutions from linearity at the low concentrations result in errors when using Eq (2) for multicomponent systems containing electrolytes.

The problem of the deviation from linearity of electrolytes can be eliminated by modifying Eq (1) to include an intercept.

$$\log \frac{a}{X_1} = K_{12} X_2^2 + b_{12} \qquad (3)$$

Assuming that the contribution of one solute to the total interaction of the intercepts b, is proportional to the fraction of that particular solute with respect to the other solutes, Eq (3) can be extended to multicomponent systems as follows:

$$\log \frac{a}{X_1} = -[(-K_{12})^{1/2} X_2^2 + (-K_{13})^{1/2} X_3^2 + \ldots]^2 + \frac{X_2}{X_2 + X_3 + \ldots} b_{12} + \frac{X_3}{X_2 + X_3 + \ldots} b_{13} + \ldots \qquad (4)$$

Eq (4) can be used even if the plot of $\log a/X_1$ vs X_2^2 does not go through the origin, or if the plot can be subdivided into several line segments. If the plot does not form one straight line, the values of K and b to be used in Eq (4) would be for that segment of the $\log_1/X_1$ vs X_n^2 plot, where the concentrations of X_n would be located. Table 2 lists the constants to be used in Eq (4) for various solutes.

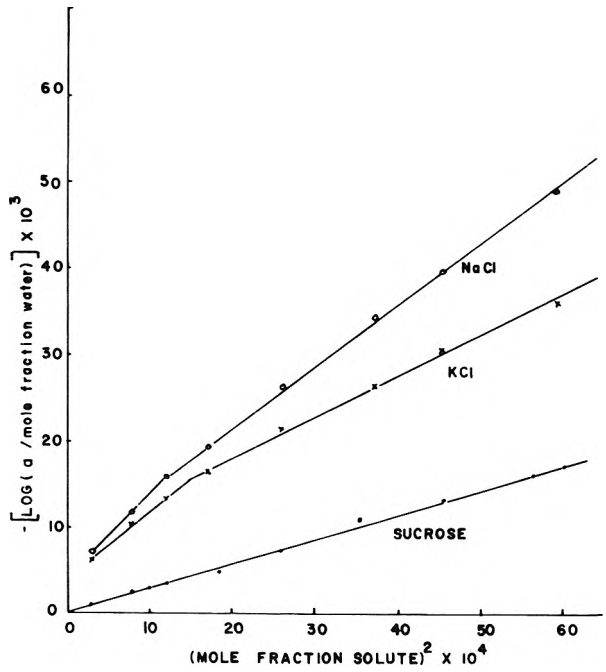

Fig. 2–Water activities of electrolyte and nonelectrolyte solutions plotted in a form that fits equation 1.

Table 1–Comparison of experimentally determined values and literature values for the water activity

Solute	Concentration (wt % solute)	Water activity: Experimental	Water activity: From Robinson & Stokes' (1959) Data
Sucrose	5.87	0.993	0.997
	15.29	0.982	0.990
	40.78	0.944	0.948
	66.29	0.839	0.856
NaCl	2.18	0.977	0.987
	10.16	0.927	0.934
	15.45	0.877	0.887
	23.41	0.799	0.795

Table 2–Constants for evaluating water activities of solutions involving various solutes using solute mole fractions

Solute	Conc Range (wt %)	Slope K	Intercept b
Sucrose	3.3 – 67.2[a]	–2.818	0
	3.3 – 68.9[b]	–2.735	0
	42 – 70.5[c]	–2.658	0.0006
NaCl	0.5 – 25.9[a]	–7.578	–0.0045
	9.5 – 25.9[a]	–7.124	–0.0073
Glycerol	0.9 – 56.3[b]	–0.505	0
	8.4 – 92[d]	–0.579	–0.0317

[a] From data reported by Robinson and Stokes (1959)
[b] From data reported by Scatchard et al. (1938)
[c] From data reported by Money and Born (1951)
[d] From data reported by Grover (1947)

The problem of assignment of the available moisture as discussed by Ross (1975) into the different solutes, is not encountered in this procedure since the mole fractions X_n are actual mole fractions with all the solutes considered. In Ross' (1975) system for calculating the water activity of mixtures, the mixture water activity is the product of the water activity of the individual solutes determined on the basis that all of the solutes are individually dissolved in an equal amount of water equivalent to the total water in the mixture.

Table 3 shows the water activity of an NaCl-sucrose-water system. All calculated values were determined using the data reported by Robinson and Stokes (1959). The experimental technique used for water activity determinations as shown in Table 1 gave water activity readings slightly lower than those reported by Robinson and Stokes (1959). The experimental values in Table 3 are also slightly lower than those calculated using Eq (4) by a similar magnitude. For comparsion, water activities calculated from Robinson and Stokes' (1959) data using Ross' (1975) technique (shown in Table 3), show slightly higher values than both the experimental and those calculated using Eq (4), particularly at lower water activities. The deviation, however, is small and it can be said that both techniques for calculating water activity are equivalent with the modified Norrish equation giving a slightly better fit at water activities lower than 0.9.

Table 3

Component in (9)			A_w			
			Calc from Eq			
Water	NaCl	Sucrose	(4)	(2)	$(A_{w_1}^o)(A_{w_2}^o)$	Experimental
60	20	20	0.740	0.741	0.759	0.744
60	15	25	0.802	0.805	0.819	0.794
60	10	30	0.861	0.865	0.873	0.846
70	15	15	0.849	0.855	0.856	0.827
70	10	20	0.897	0.902	0.900	0.879
80	10	10	0.923	0.929	0.920	0.890
80	5	15	0.954	0.961	0.955	0.963
90	4	6	0.972	0.979	0.972	0.968
90	3	7	0.976	0.983	0.977	0.973
90	2	8	0.981	0.987	0.983	0.976

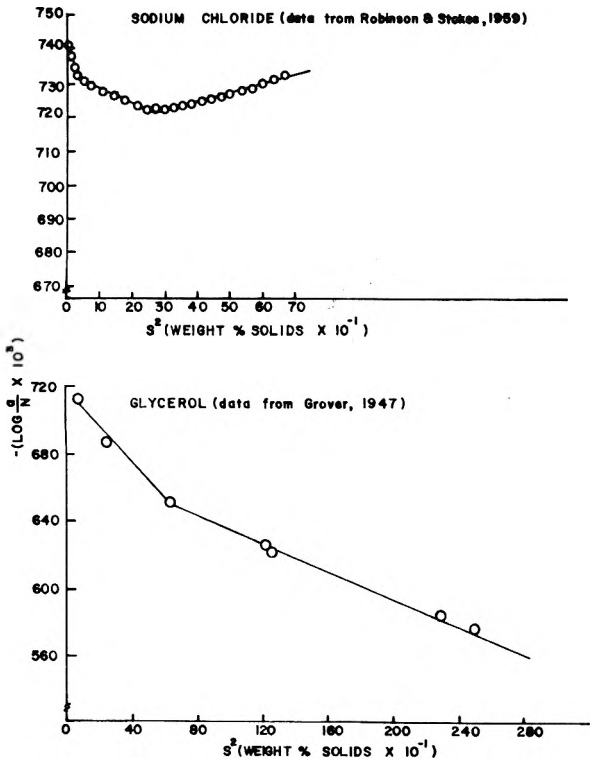

Fig. 3—Water activities of glycerol and NaCl plotted in a form that fits Eq (6).

The calculated values of water activity using Eq (4) shows an improvement over that calculated using Eq (2). Although the difference is slight in this particular example where NaCl is the only electrolyte present, the effect would be more apparent when mixtures of several electrolytes are present.

The concept of including the intercepts in equations for predicting the water activity such as that used in Eq 3 and 4, is useful for electrolytes and other materials where the plots of the water sorption isotherm in the form that fits Eq (1) consists of one or more line segments that do not pass through the origin. The technique is strictly empirical but it gives a very good fit with experimental data.

Equations for calculating water activities of systems containing a solid phase

Eq (1) through (4) are all based on mole fractions and therefore would be suitable only for solutes having known molecular weights. For most food systems, there would be complex molecules present, and the assignment of molecular weights to these to calculate the mole fraction would not be possible. Eq (1) can be modified for use with systems containing complex molecules as follows:

$$\text{Log}\frac{a}{N_1} = K\left(\frac{S_2}{M_2}\right)^2 + b \qquad (5)$$

where: N_1 = moles water/100g solution; S_2 = g solute/100g solution; M_2 = molecular weight of solute. Eq (5) is similar to Eq (1) except that the basis is 100g of solution rather than 1 mole of solution. Since M is constant for solids in a given system, it could be combined with K for a constant characteristic of that system, and Eq (5) becomes:

$$\log\frac{a}{N_1} = K S_2^2 + b \qquad (6)$$

Figures 3 and 4 show plots of water activities of various materials in a form that fits Eq (6). The plots do not show single straight lines. However, since both the slopes and intercepts are used in Eq (6), water activities at any concentration can still be calculated.

The dip in the sorption isotherms for NaCl and for starch, dehydrated meat, and wheat flour could be due to varying extent of solute-water interactions occurring at various solids contents. The isotherms for electrolytes like KCl and H_2SO_4 all show similar patterns as that for NaCl, whereas nonelectrolytes like dextrose and glycerol follow the same pattern as that of sucrose. Although the isotherms for starch, dehydrated meat, and wheat flour show what appears to be an uncharacteristic dip when plotted in the form that fits Eq (6), a reconstructed isotherm (g H_2O/g dry matter vs a_w) calculated from the constants reported in Table 4 for these materials, would show a standard "S" shaped isotherm.

The isotherm for starch, dehydrated meat, and wheat flour follow a similar trend as that reported by Shanbhag et al. (1970) when they plotted wide-line Nuclear Magnetic resonance absorption by flour samples in an RF saturated field

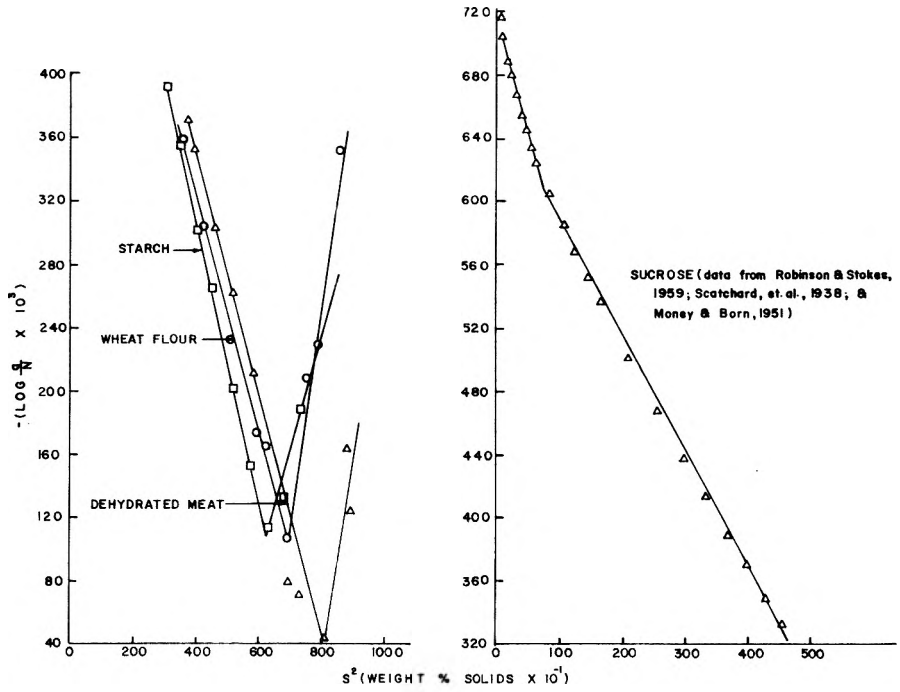

Fig. 4—Water activities of sucrose solutions and sorption isotherms of some solids plotted in a form that fits Eq (6). The data for starch, wheat flour, and dehydrated meat were determined using the manometric technique at 25° C.

against water content. These authors showed that in an RF saturated field, the area under the derivative curve for NMR absorption was zero for pure water, whereas with water "bound" to solids, the area was directly proportional to the quantity of water present. The shape of the isotherms in Figure 4 and the data scatter at the apex of the dip is very similar to the NMR absorption vs moisture content curve. Thus, solute water interactions is probably the main reason for the changes in the slope and direction of the isotherms when plotted according to Eq (6).

Values of the constants for various solids to fit Eq (6) are shown in Table 4. Similar constants can be calculated for other solids and solutes if their sorption isotherms are known. The values in Figure 4 were determined by calculating the equation of regression lines through data points within concentration ranges where the points form a straight line.

Equations for calculating water activities of multicomponent solid systems containing complex molecules

The real value of Eq (6) would be in determining the water activity of mixtures of solids. When solids are mixed, there would be migration of moisture from one component to the other until they finally come into equilibrium. The development of the equation for calculating water activities of solid mixtures from the sorption isotherms of individual components, involve the following considerations:

(a) After equilibration, components 1 and 2 would contain W_{e1} and W_{e2} g water/100g dry matter, respectively. An expression relating W_{e1} and W_{e2} can be derived from the proportions of components 1 and 2 and the initial moisture contents W_{i1} and W_{i2} by a moisture balance.

(b) If component 1 has the moisture content W_{e1} it would have a water activity a_1. A similar argument applies to component 2. At equilibrium, the water activities of all phases are equal.

Considering component 1, substituting appropriate terms to Eq (6) to correspond to the initial and equilibrium conditions, and subtracting one form of Eq (6) from the other, the following equation can be obtained:

$$\log \frac{a_{i1}}{N_{i1}} - \log \frac{a_{e1}}{N_{e1}} = K_{i1} S_{e1}^2 + b_{i1} - K_{e1} S_{e1}^2 - b_{e1} \qquad (7)$$

If the conditions are such that moisture contents in the

Table 4—Constants of some solutes and solid mixtures used for evaluating water activities at 25° C

Solid	Concentration (wt % solids)	Slope k	Intercept b
Sucrose	0 – 23.5[a]	0.000167	–0.7219
	23.5 – 67.8[a]	0.00007485	–0.6648
NaCl	0 – 7.5[a]	0.0002063	–0.7417
	7.5 – 17.3[a]	0.00002975	–0.7308
	17.3 – 26.4[a]	–0.00003039	–0.7118
Glycerol	0 – 25[b]	0.000104	–0.7165
	25 – 53[b]	0.00002814	–0.6552
Starch[c]	50 – 79.1	0.0000892	–0.6672
	79.1 – 95	–0.0000649	0.2983
Dehydrated meat[c]	50 – 89.8	0.0000805	–0.6705
	89.8 – 95	–0.000123	0.9466
Wheat flour[c]	50 – 83.2	0.0000749	–0.6269
	83.2 – 100	–0.0001937	1.2543

[a] Calculated from data reported by Robinson and Stokes (1959)
[b] Calculated from data reported by Grover (1947)
[c] Calculated from author's data from these materials determined using the vapor pressure manometric technique.

Table 5–Calculated and experimental water activities of solid mixtures

Mixture	Component	Wt in grams	% H_2O Wet basis	Water activity: Calc Eq (13)	Water activity: a_1^o (a_2^o)	Water activity: Experimental
1	Starch	50	14.9	0.467	0.783	0.444
	Wheat	50	11.44			
2	Starch	30	14.9	0.425	0.720	0.428
	Wheat	70	11.44			
3	Starch	70	14.9	0.491	0.738	0.460
	Wheat	30	11.44			
4	Starch	50	22.45	0.845	0.938	0.879
	Wheat	50	18.76			
5	Starch	50	28.6	0.927	0.987	0.965
	Wheat	50	26.5			
6	Starch	20	28.6	0.901	0.946	0.941
	Wheat	80	26.5			

range from W_{i1} to W_{e1} all fall in the same straight line, Eq (7) can be simplified to:

$$\log \frac{a_{i1} N_{e1}}{a_{e1} N_{i1}} = K_1 (S_{i1}^2 - S_{e1}^2) = K_1 (S_{i1} + S_{e1})(S_{i1} - S_{e1}) \quad (8)$$

Expressing S in terms of W and substituting in Eq (8):

$$S_{i1} = \frac{100 \times 100}{100 + W_{i1}}; S_{e1} = \frac{100 \times 100}{100 + W_{e1}}$$

$$\log \frac{a_{e1} N_{e1}}{a_{e1} N_{i1}} = K_1 \times 10^8 \left(\frac{1}{100 + W_{i1}} - \frac{1}{100 + W_{e1}} \right) \times \left(\frac{1}{100 + W_{i1}} + \frac{1}{100 + W_{e1}} \right) \quad (9)$$

Simplifying:

$$\log \frac{a_{i1} N_{e1}}{a_{e1} N_{i1}} = K_1 \times 10^8 \frac{(W_{e1} - W_{i1})(200 + W_{e1} + W_{i1})}{(100 + W_{e1})^2 (100 + W_{i1})^2}$$

Solving for $(W_{e1} - W_{i1})$:

$$(W_{e1} - W_{i1}) = \frac{(100 + W_{e1})^2 (100 + W_{e1})^2}{K \times 10^8 (200 + W_{e1} + W_{i1})} \log \frac{a_{i1} N_{e1}}{a_{e1} N_{i1}} \quad (10)$$

A similar procedure can be employed on component 2 and:

$$(W_{i2} - W_{e2}) = \frac{(100 + W_{i2})^2 (100 + W_{e2})^2}{K_1 \times 10^8 (200 + W_{e2} + W_{i2})} \log \frac{a_{e2} N_{i2}}{a_{i2} N_{e2}} \quad (11)$$

A moisture balance between components 1 and 2 and the mixture would give:

$$\frac{D_1 (W_{e1} - W_{i1})}{100} = \frac{D_2 (W_{i2} - W_{e2})}{100} \quad (12)$$

Substituting Eq 10 and 11 into Eq 12 and substituting a for a_{e1} and a_{e2}, the following equation for calculating the water activity is obtained:

$$\log a = \frac{\left(\frac{1}{(D_1)(100 + W_{i1})^2} + \frac{(D_2)(100 + W_{i2})^2}{(K_2)(200 + W_{e2} + W_{i2})} \right)}{(K_1)(200 + W_{e1} + W_{i1})} \times \left(\frac{D_1 (100 + W_{i1})^2}{K_1 (200 + W_{e1} + W_{i1})} \log \frac{a_{i1} N_{e1}}{N_{i1}} + \frac{D_2 (100 + W_{i2})^2}{K_2 (200 + W_{e2} + W_{i2})} \log \frac{a_{i2} N_{e2}}{N_{i2}} \right) \quad (13)$$

Everything is known in Eq 13 except for W_{e1} and W_{e2} and consequently N_{e1} and N_{e2}. A simplyfying assumption that would allow the use of Eq 13 in calculating water activities of solid mixtures is that W_{e1} and W_{e2} are equal. These values can then be calculated using Eq 12. N_e and W_e can be calculated as follows:

$$N_e = \frac{W_e \times 100}{(100 + W_e)(18)}; W_e = \frac{D_1 W_{i1} + D_2 W_{i2}}{D_1 + D_2}$$

The error introduced by this simplyfying assumption would be very small since terms in Eq 13 that involves W_{e1} are added on to other terms which may have a magnitude larger than W_{e1} thus minimizing the effect of the error. The error contributed by N_e would tend to cancel out between component 1 and component 2 since a low value for one would be compensated for by a high value in the other upon addition of the two terms involving N_e in Eq 13.

To illustrate the use of Eq 13, consider a mixture of 70g starch (component 1, 14.9% moisture, a = 0.557) and 30g of wheat (component 2, 11.44% moisture, a = 0.345).

D_1 = 70 (0.851) = 59.57g dry solids
D_2 = 30 (0.8856) = 26.57g dry solids
W_{i1} = 14.9/(1 – 0.149) = 17.51g H_2O/100g dry solids
W_{i2} = 11.44/(1 – 0.1144) = 12.92g H_2/100g dry solids

$$W_e = \frac{59.57 (17.51) + 26.57 (12.92)}{59.57 + 26.57} = 16.094 \frac{\text{g } H_2O}{\text{100g dry solids}}$$

$$N_e = \frac{16.094 (100)}{116.094 (18)} = 0.77 \text{ moles } H_2O/\text{100g wet solids}$$

From Table 4, the values of constants for starch (79.1–85.1% solids) are: $K_1 = -0.000065$, and $b_1 = 0.298$. For Wheat (83.2–93.3% solids); $K_2 = -0.000194$, and $b_2 = 1.254$. Using Eq 6, for $S_1 = 85.1$ and $S_2 = 88.6$g dry solids/100g of each component; $a_{i1}/N_{i1} = 0.671$ and $a_{i2}/N_{i2} = 0.538$. Substituting in Eq 13, the calculated value of a = 0.491. This compares with an experimentally measured water activity for this mixture of 0.460.

Using Ross' (1975) technique for calculating the water activity, the water activity of the starch phase if all the water present is assumed to be in starch (18.9g H_2O/100g starch), would be $a_1^o = 0.779$. The water activity of the wheat phase assuming all the water present to be in wheat (34.29g H_2O/100g wheat), would be $a_2^o = 0.948$. The water activity of the mixture would be $(a_1^o)(a_2^o) = 0.770\ (0.948) = 0.738$. If the individual water activities are taken for the original components, the calculated water activity would be 0.557 (0.345) = 0.192. There is an insignificant amount of soluble solids present in this system for solute-insoluble solids interaction to

possibly account for the discrepancy. The problem of moisture assignment in systems containing insoluble solids has been mentioned by Ross (1975) in his report.

Calculation of the water activity using Eq (13) comes closest to predicting the water activity of mixtures containing a high concentration of insoluble solids, compared to other techniques previously reported. Table 5 lists water activities of various mixtures calculated using Eq 13 and determined experimentally. Water activities calculated using Ross' (1975) technique are close to experimentally determined values only at water activities above 0.9, as shown for mixtures 5 and 6.

The concept of equal phase water activity at equilibrium was previously used by Salwin and Slawson (1959) for mixtures of dried foods. The major limitation of this technique is the assumption of a linear isotherm and the calculated values depend very largely on how the tangents to the isotherms are drawn to obtain the slopes used in the equation for calculating the equilibrium water activity. In the example above, a calculated A_W ranging from 0.48–0.51 was obtained using Salwin and Slawson's technique depending upon how the tangent to the isotherms were drawn. The problem becomes more acute when the initial moisture contents fall in the region of the isotherm where the slope changes rapidly with change in moisture content.

Expressing the isotherm in the form of Eq 6 gives a wider range of moisture content where the isotherm is given. Eq 13 appears to be unwieldy but it gives an accurate estimate of the equilibrium moisture contents of solid mixtures particularly at low water activities. The limitations of Eq 13 are that it is limited to two components, the range of initial to final moisture contents must fall with the same line segment in the water activity-composition plot for each component, and the equation itself is too unwieldy to apply for a mixture containing more than two components. One of these limitations can be circumvented. When a mixture of two solids is considered, the initial moisture contents can be reallocated between the two components such that upon reaching equilibrium, both ranges of initial and final moisture contents fall within a single line segment in the sorption isotherm plot. Using this technique, Eq 13 can be used for calculating equilibrium water activities of any mixture to two solids. If both sorption and desorption isotherms are available, the constants should be derived from the corresponding isotherm.

Eq 13, however, made possible experimental verification of the validity of the concepts used in its derivation. The concept of equality of phase water activity between phases at equilibrium in systems where distinct phases exist, allows a fresh insight into the role of insoluble solids or soluble solids at concentrations close to saturation, in influencing the overall water activity of a system.

SYMBOLS

a = water activity
X = mole fraction
K = constant = slope
b = constant = intercept
N = number of moles water/100g mixture
M = molecular wt
S = wt dry solids/100g mixture
D = actual weight of dry material
W = water content in g H_2O/100g dry matter

Subscripts

1, 2 . . . n indicates component 1, 2 . . . etc.
12, 13 . . . 1n indicates between component 1 and 2, 1 and 3, . . . etc.
i . . . initial
f . . . final
e . . . equilibrium

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Ms received 6/16/75; revised 12/13/75; accepted 12/16/75.

Presented at the 35th Annual Meeting of the Institute of Food Technologists, Chicago, Ill., June 8—12, 1975.

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PROTEIN-STARCH EXTRACTION AND NUTRITIVE VALUE OF THE BLACK-EYED PEA (Vigna sinensis) AND ITS PROTEIN CONCENTRATES

ABSTRACT

Two processes were developed for the preparation of protein concentrates with a concommitant recuperation of the starch fraction from the Black-eyed pea (*Vigna sinensis*). The first was a one-stage extraction process using a 4:100 peeled meal:solvent ratio, a pH of 9.0, and 25°C, and the second a two-stage process of 1 hr per stage using a peeled meal:solvent ratio of 12:100, pH of the suspension 6.8, and 25°C. Protein extraction efficiencies of 87 and 86% for the one- and two-stage processes, respectively, were not improved by increasing either extraction time or temperature (up to 50°C). The starch recuperation was 84% in the former and 76% in the latter. Both protein concentrates (66–70% protein) obtained by isoelectric point precipitation proved to have a higher methionine and cystine content and a higher PER value than the original peeled meal. Preliminary results indicate the suitability of the protein concentrates for use in the formulation of pasta products, sausages and tacos.

INTRODUCTION

ALTHOUGH approximately 13,000 species of legume seeds have been identified, only about 20 are of relative economic importance as a food item in the Latin American Region. In Central America and Mexico the number is even smaller.

Among the legume seeds which are either not consumed or consumed at low levels, and only in specific areas, is the Black-eyed pea (*Vigna sinensis*). This legume can be grown relatively easily, producing high yields in regions of low altitude that are inadequate for the growth of other legume seeds such as *Phaseolus vulgaris* (Rachie, 1973). Thus, as happens with other legume seeds like the Jack bean (*Canavalia ensiformis*) that have similar agricultural characteristics (Rachie, 1973), the Black-eyed pea has a high potential in zones of varying climates and altitudes. This is the case for most Latin American areas, where it would not compete with the common bean (*P. vulgaris*) and still could provide an additional protein source.

The fact that the Black-eyed pea is not used as a human food can be attributed mainly to regional dietetic habits that do not include this legume as part of the common diet. Hence, in order to increase its utilization as a protein source in the area, different possibilities for its industrialization need to be developed. Nutritionally, the introduction of the Black-eyed pea in the human diet of Latin America would be desirable, since such legume has been shown to have a much lower concentration of hemagglutinins and trypsin inhibitor than the red or black beans (*P. vulgaris*) that constitute a common staple (Bressani and Elías, 1974; Hernández Infante, 1975).

A relatively high starch content has been reported for this and other legume seeds (Bressani and Elías, 1974), and a simple process has been designed for the combined protein and starch isolation from the Mungo bean (*P. mungo*) (Altschul, 1966; Bhumiratana and Nondasuta, 1971) and the Jack bean (*Canavalia ensiformis*) (Molina and Bressani, 1975). We therefore considered it worthwhile to investigate the possibility of its industrial utilization. Recently, Satterlee et al. (1975) reported protein extraction from the Great Northern bean (*P. vulgaris*) by a dilute saline extraction method, and indicated that the protein isolate obtained has desirable functional properties which leave no doubt as to its possible inclusion in food systems.

The present work was undertaken to establish optimum conditions for extraction and recovery of the protein and starch from the Black-eyed pea, through a simple, low-cost process. A second purpose was to determine the nutritional characteristics of the Black-eyed pea and of the protein concentrates obtained thereof.

MATERIALS & METHODS

THE BLACK-EYED PEA used in this study was grown in the low-lands of Guatemala at an altitude of 213m above sea level. It was planted in August and harvested in December 1973.

For the extraction trials, the Black-eyed pea was peeled through air separation of the seed coat after coarse milling of the seed. The meat thus obtained was milled in a hammer mill equipped with a 30-mesh screen, and the resulting meal was used throughout the extraction experiments. Preliminary extraction trials were carried out to determine the minimum time and pH conditions for maximum extraction yields. Then, a factorial design (3 × 3 × 3), temperature (25, 35 and 50°C), pH (6.8, 8.0 and 9.0) and meal-to-solvent ratio (4:100, 8:100 and 12:100) using an extraction time of 1 hr was adopted to determine the extracting conditions to be used at pilot plant scale.

At the laboratory level, the extraction experiments were carried out in triplicate using a reciprocal water-bath shaker (New Brunswick Scientific, Model R-76) with constant agitation equivalent to 120 strokes per minute. The recuperation of the protein extracts (supernatant) was carried out by centrifuging at 1,100 × G for 20 min. The starch remained as the residue from the centrifugation process. The NaOH and HCl used to adjust the pH were all reagent grade. The protein concentrate was obtained through isoelectric point precipitation; it was then washed for a minimum of three times with water adjusted to a pH equivalent to the isoelectric point and dried under vacuum at 60 ± 1°C for 36 hr prior to further analysis.

When a multiple stage extraction system was studied, the residue was resuspended in an amount of fresh solvent calculated to give the same meal:solvent ratio as in the previous stage. This calculation was based on the total solids extracted in the aforementioned stage.

The pilot plant-scale protein extraction experiments were carried out in a Lee 40 gal jacketed kettle with continuous agitation (60 rpm), equipped with a constant extraction temperature control. The centrifugation steps at the pilot plant level were performed in a basket centrifuge (Watson, Laidlaw & Co., Ltd., Glasgow, Scotland) at 1,000 rpm. The centrifuge was equipped with a 200-mesh screen. The protein concentrate obtained was air dried in a convection oven (Precision Scientific, Model 625) at 60 ± 1°C (wet bulb temperature, 27 ± 1°C) for 24 hr prior to further analysis. The material was placed on the trays in layers of about 0.75 cm depth.

Nitrogen, ash, ether extract, moisture, crude fiber, total solids and starch were determined in duplicate according to the AOAC (1970). Protein was calculated multiplying the nitrogen content by the custom-

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ary conversion factor 6.25. Total soluble sugars were determined in the alcohol washings of the starch analyses, by the phenol-sulfuric method according to Dubois et al. (1956), using glucose as standard.

The amino acid analyses were done in duplicate using an amino acid autoanalyzer and following the method of Spackman et al. (1958). Cystine and tryptophan were determined microbiologically using Difco media (Difco Laboratories, Detroit, Mich.) and *Leuconostoc mesenteroides* P-60 and *Lactobacillus arabinosus* 17-5, respectively.

The protein efficiency ratio (PER) was evaluated essentially by the AOAC method (1970). Weanling rats of the Wistar strain from the INCAP animal colony were distributed in groups of four males and four females each. All diets were supplemented with a 4% salt mixture (Hegsted et al., 1941), 5% cottonseed oil, 1% cod liver oil and enough corn starch to adjust to 100g; 5 ml of a vitamin B solution (Manna and Hauge, 1953) were added. The materials were not subjected to any thermal cooking treatment prior to this test.

RESULTS & DISCUSSION

THE SEED COAT was found to represent 11.43 ± 2.64% (w/w) of the whole Black-eyed pea. The percent composition (on "as is" basis) of the 30-mesh whole and peeled Black-eyed pea meals is shown in Table 1. As the data reveal, the peeled Black-eyed pea meal (88.57 ± 2.26% of the whole seed) is particularly rich in protein (24.36%) and starch (47.18%). In fact, the peeled meal constitutes 97.6 and 93.7% of the total protein and starch content of the whole meal, respectively. On the other hand, the peeling operation represents a 68.6% reduction in the total fiber content of the whole meal. Therefore, we consider that using the peeled Black-eyed pea meal would not affect the total protein-starch extraction yields; furthermore, it would facilitate the final purification of the starch.

Preliminary extraction experiments using a 5:100 meal-to-solvent ratio, a pH of 6.8, and 25°C as extracting conditions, indicated that the maximum protein extraction yield (71%) occurred in a minimum of 1 hr. This extraction time, therefore, was adopted for all subsequent experiments. Similar results have been previously reported by Molina et al. (1974) and by Molina and Bressani (1975) in their experiments with Jack bean (*C. ensiformis*).

The pH effect on the protein extraction yields was studied varying the pH of the aqueous suspension by the addition of NaOH and/or HCl prior to the extraction, which was carried out at 25°C during 1 hr using a meal-to-solvent ratio of 5:100. The results are shown in Figure 1. As may be observed, the protein extraction is favored either by an acid pH (below 3.0) or by a pH over 5.0, results which agree with those reported for several legume seeds (Bressani and Elías, 1974; Evans and Kerr, 1963; Hang et al., 1970a; Pant and Tulsiani, 1969; Molina and Bressani, 1975; Molina et al., 1974). Practically no pH drift was observed during the protein extraction treatment. Analysis of the data demonstrated that the difference between the extraction efficiency obtained at a pH of 8.0 was not statistically significant ($P < 0.05$) from that obtained at pH values up to 11.0. The low protein solubility at the pH values of 3.0–5.0 may be attributed to the intermolecular attraction of proteins in the isoelectric zone. The pH of minimum protein solubility was found to be 4.0. Therefore, this pH value was used for the recuperation of the protein concentrates.

The effect of the meal-to-solvent ratio on the protein extraction efficiency at 25°C, with a pH of 6.8 for 1 hr as extracting conditions, is shown in Figure 2. As expected, the extraction efficiency was found to be inversely related to the meal-to-solvent ratio used, results which are in accordance with the findings reported for other legume seeds (Hang et al., 1970b, c; Molina et al., 1974; Molina and Bressani, 1975).

Using a factorial design (3 X 3 X 3), temperature (25°C, 35°C and 50°C), pH (6.8, 8.0 and 9.0), a meal-to-solvent ratio (4:100, 8:100 and 12:100) and 1 hr extraction time, it was found that while the last two variables exerted a significant ($P < 0.05$) effect on the protein extraction efficiency, the effect of temperature was not statistically significant ($P < 0.05$). Consequently, a temperature of 25°C (practically ambient temperature) was applied in all subsequent experiments. Neither a temperature higher than 50°C nor a pH above 9.0 were evaluated during these experiments, bearing in mind that they could sacrifice both the recovery yields and the quality of the starch.

Based on the above results, two pilot plant-extraction runs were carried out. One was a one-stage extraction at 25°C, a pH of 9.0 and 4:100 meal-to-solvent ratio; the other was a two-stage extraction at 25°C, a pH of 6.8 (equivalent to that of the aqueous suspension of the material) and a meal-to-solvent ratio of 12:100. The main pilot plant operations and material balance data obtained for the one- and two-stage processes evalu-

Table 1—Percent composition of the whole and peeled Black-eyed pea meals

Component	Black-eye pea meal	
	Whole	Peeled
Moisture	13.38	12.90
Ether extract	1.92	1.92
Ash	4.13	4.20
Crude fiber	4.93	1.75
Protein (N X 6.25)	22.10	24.36
Nitrogen free extract	53.54	54.87
Starch	44.58	47.18
Total soluble sugars[a]	6.66	6.80

[a] Expressed as glucose

Table 2—Amino acid composition of the peeled Black-eyed pea meal and of the Black-eyed pea protein concentrates obtained through the one- and two-stage extraction systems evaluated (g/16g N)

Amino acid	Black-eyed pea peeled meal	Protein concentrates		
		I[a]	II[b]	III[c]
Alanine	5.55	4.79	4.52	5.01
Aspartic acid	14.55	14.66	12.48	13.60
Glutamic acid	22.56	21.38	19.18	20.89
Glycine	5.30	4.42	4.16	4.28
Serine	2.45	3.96	3.83	4.26
Tyrosine	3.10	3.93	3.57	4.04
Arginine	8.06	8.57	7.90	8.18
Histidine	3.90	3.34	3.56	3.56
Isoleucine	5.45	4.88	5.51	6.16
Leucine	9.99	10.12	9.33	9.91
Lysine	8.58	7.75	7.70	8.15
Methionine	1.08	1.52	1.41	1.26
Phenylalanine	6.87	7.57	7.13	7.42
Valine	6.96	6.99	6.46	6.94
Threonine	2.97	4.19	3.34	3.79
Cystine	0.27	0.35	0.32	0.32
Tryptophan	0.95	1.02	0.98	1.00
Chemical score	30	40	39	33

[a] Protein concentrate from the first extraction step of the two-stage system at a pH of 6.8.
[b] Protein concentrate from the second extraction step of the two-stage system at a pH of 6.8.
[c] Protein concentrate from the one-stage extraction system at a pH of 9.0

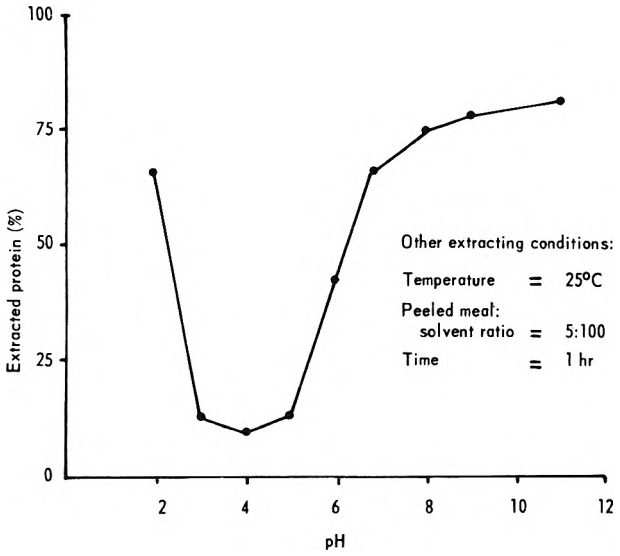


Fig. 1—Effect of pH on protein extraction efficiency.

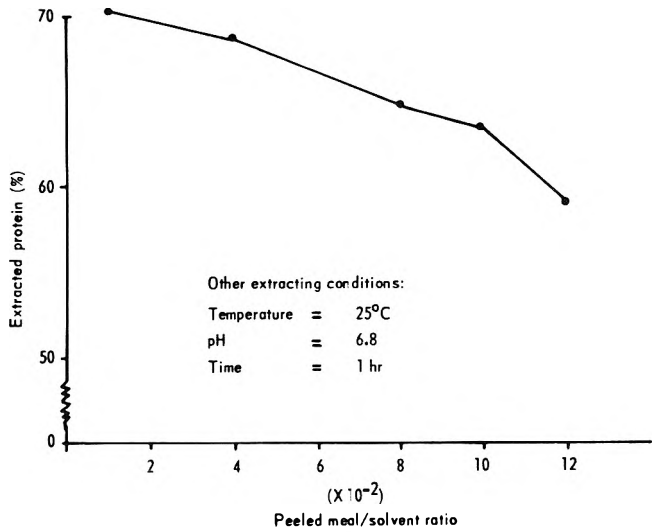
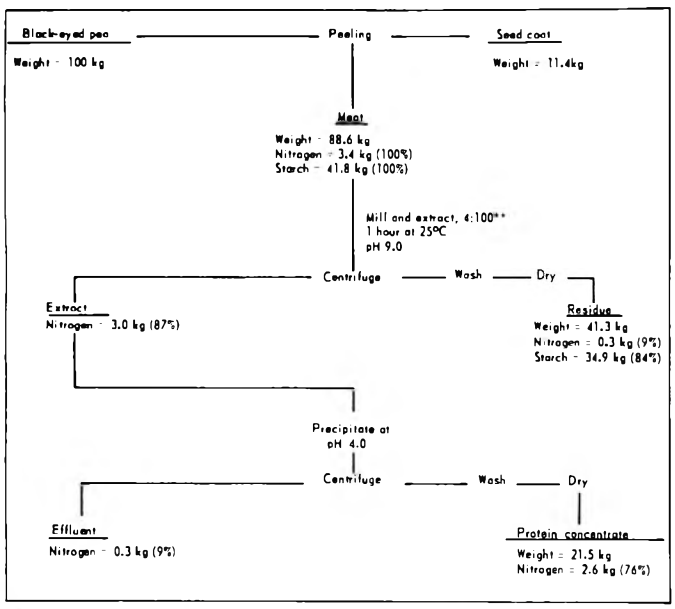


Fig. 2—Effect of the peeled meal-to-solvent ratio on protein extraction efficiency.

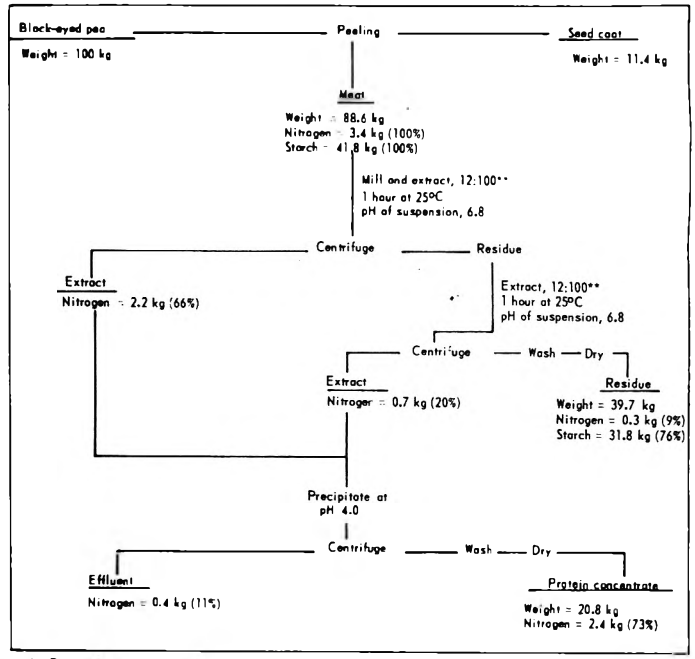
ated are shown in Figures 3 and 4, respectively. As the data reveal, the protein extraction efficiency obtained through both processes was very similar (87 and 86%, respectively). Another important finding was that the total protein recuperated from the protein concentrates obtained by both systems, was very similar. The final protein concentrates had a protein content fluctuating between 66 and 70%. However, the starch recuperation through the one-stage extraction system (84%) was significantly ($P < 0.05$) higher than that obtained by the two-stage system (76%). This difference may be attributed to the larger possibility of residue (starch) losses when using the two extraction procedures than when applying only the one-stage operation. The use of a 12:100 meal-to-solvent ratio in the two-stage extraction system did not compromise the operations during both the extraction and recuperation processes.

Table 2 shows the amino acid composition of the original peeled Black-eyed pea meal and of samples of the protein concentrates obtained from the one-stage process as well as



* Figures in parenthesis indicate percentage of nitrogen or starch from the original peeled meal.
** Peeled meal: solvent ratio.

Fig. 3—Balance of materials obtained from the protein-starch one-stage extraction process at a pH of 9.0.*



* Figures in parenthesis indicate percentage of nitrogen or starch from the original peeled meal.
** Peeled meal: solvent ratio.

Fig. 4—Balance of materials obtained from the protein-starch two-stage extraction process at a pH of 6.8.*

Table 3—Performance of rats fed the peeled Black-eyed pea flour and the Black-eyed pea protein concentrates obtained in the pilot plant runs

Material fed	Protein in diet (%)	Avg wt gain (g)	Protein efficiency ratio (PER)
Peeled meal	9.8	56.8	1.43 ± 0.28*
Protein conc I[a]	10.2	57.0	1.56 ± 0.31
Protein conc II[b]	9.6	58.3	1.86 ± 0.33
Casein	9.2	73.6	2.42 ± 0.46

[a] Protein concentrate from the two-stage extraction system at a pH of 6.8.
[b] Protein concentrate from the one-stage extraction system at a pH of 9.0
* Standard deviation of the mean

from each of the two extracts of the two-stage system. Very little variation was obtained in the duplicate samples run. As may be observed, no destruction of cystine was detected in the sample obtained at pH 9.0. It is of interest to notice that the methionine content of the original peeled meal was always lower than those obtained for any of the protein concentrates studied. The chemical score determination (Pike and Brown, 1967) using the FAO protein reference pattern (FAO, 1973) as standard, revealed that methionine was the most limiting essential amino acid in all materials. The protein score was 30 for the original peeled Black-eyed pea meal, while the one-stage (pH of 9) protein concentrate rendered a value of 33; a value of 40 and 39 was attained for the protein concentrate samples of the first and second stages of the two-stage process at pH 6.8, respectively.

The leucine:isoleucine ratio of the original material (1.83) was higher than that resulting from the one-stage protein concentrate (1.61) and for each of the protein concentrates obtained in the two-stage process (1.72 and 1.69, respectively). The lower value presented by the protein concentrates when compared to the original material could probably favor their amino acid balance. No peak of a lysine-alanine complex was detected in the amino acid chromatogram of any of the protein concentrates.

The growth and PER results obtained with the original meal and the protein concentrates obtained by the one- and two-stage processes evaluated, are summarized in Table 3. Data for the casein standard protein are also included. These findings indicate that the nutritive value of the protein concentrates was always higher than that of the original meal protein. This can be attributed to the higher methionine content of the protein concentrates when compared to the original material (Table 2). The possibility that the presence of antinutritional factors in the peeled uncooked Black-eyed pea meal could be affecting the aforementioned results was discarded, since as reported by Hernández Infante (1975), the PER of the Black-eyed pea used in the present study was 1.44, 1.33 and 1.33 when subjected to 15, 30 and 45 min cooking at 121°C. Furthermore, the same author indicates that the antitryptic factor concentration in the raw Black-eyed pea is very similar to that found in cooked black, white, or red beans (around 5 T.U.I.). The fact that analysis of the data showed that only the protein concentrate derived from the one-stage extraction system (pH of 9.0) had a significantly ($P < 0.05$) higher PER value than that of the original material, indicates a possible better amino acid balance in this protein concentrate than in that resulting from the two-stage extraction system. Such a possibility seems to be supported by the values reported above for the leucine:isoleucine ratios.

Preliminary experiments revealed that both protein concentrates obtained in this study are suitable for inclusion at a 10 or 15% level in the formulation of conventional foods such as pasta products. This observation is of interest, since legume proteins have been shown to complement cereal proteins in pasta, yielding a final product of improved protein quality (Molina et al., 1975). On the other hand, both protein concentrates were found suitable for inclusion in the formulation of sausages and tacos.

We consider that the findings reported herein indicate that the Black-eyed pea, a legume presently consumed in negligible quantities as a food item in Latin America, but that grows easily producing high yields (Rachie, 1973), could be used in the production of protein concentrates and starch. The protein concentrates, in turn, could be utilized in the production of several conventional food formulations or, possibly as well, in the production of texturized food items without the problems of residual hemagglutinin or trypsin inhibitor activity.

Conscious of the difficulties arising from the implementation of a wet process as the one described here for a protein-starch extraction system, we are currently investigating the possibility of effecting the separation of the above-mentioned nutrients by means of a dry process. At present the economics of the wet process described in this paper are being evaluated as well. Preliminary data indicate that the products obtained, namely protein concentrates and starch, could compete favorably with similar counterparts (soybean protein concentrates and corn starch) in the local market. However, more data (agricultural production costs, etc.) are still needed for Black-eyed pea in these countries for arriving at a final cost figure. Such data are not readily available, primarily because at present Black-eyed pea is not a commercial crop in the Central American countries, mainly due to the prevailing regional dietetic habits, which do not include this legume as part of the common diet.

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Presented at the 35th Annual Meeting of the Institute of Food Technologists, Chicago, Ill., June 8—12, 1975.

This research was carried out with funds from the Research Corporation, New York, N.Y. (Grant-in-aid INCAP No. 740.)

Data taken from the thesis submitted by C.E. Argueta to the Centro de Estudios Superiores en Nutrición y Ciencias de Alimentos (CESNA), University of San Carlos de Guatemala/INCAP, in partial fulfillment of the requirements for the M.S. degree.

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INFLUENCE OF WATER CONTENT ON THE STABILITY OF MYOGLOBIN TO HEAT TREATMENT

ABSTRACT

The effect of heat treatment on a model protein, sperm whale myoglobin, at various water contents has been investigated at neutral pH. Samples ranging from solid preparations of 3% water content to dilute solutions of 99.6% water content have been examined. Differential scanning calorimetry (DSC) has been used for the heat treatments as well as for analyzing the effect of the heat treatments on the protein samples. At water contents below 30% only a certain fraction of the heat-treated protein sample underwent irreversible transitions. This fraction of the protein sample increased linearly with the amount of water present during the heat treatment. Above 30% water content the entire protein sample underwent irreversible transitions. The heating rate was shown to influence the transition temperature as well as the apparent transition heat. Possible applications in food technology and food processing of the DSC technique used are discussed.

INTRODUCTION

HEAT TREATMENT is an important part of food processing and also of the production of protein concentrates and isolates. As these processes are often accompanied by an undesirable protein denaturation, much attention has been paid to the study of these effects. For example, the influence of heat processing on the functionality of plant proteins has recently been reviewed by Wu and Inglett (1974).

It has previously been observed that the presence of water plays an important role in determining the effect of a heat treatment on protein samples (Barker, 1933; Pence et al., 1953; Bull and Breese, 1968). It has also been observed that heating during a long period of time can result in similar effects as heating at a higher temperature during a shorter period of time (Barker, 1933; Mann and Briggs, 1950; Pence et al., 1953).

The effect of the heat treatment has been expressed as either a loss of dispersibility (Mann and Briggs, 1950) or as a loss of solubility (Barker, 1933; Pence et al., 1953). As these investigations dealt with complex preparations of proteins, conclusions on a molecular level were rather uncertain. Differential scanning calorimetry (DSC), recently described for studying the conformational transitions of poly-γ-benzyl-L-glutamate (Simon and Karasz, 1974) allows the simultaneous determination of the transition temperature (T_m) and the transition heat (ΔH_{app}). A similar technique, differential enthalpic analysis (DEA) was originally used by Crescenti and Delben (1971) for the evaluation of enthalpies of thermal denaturation for chymotrypsinogen A, lysozyme and ribonuclease A.

The intention of the present investigations was to study the effect of heat treatments by DSC technique on a well-known model protein, sperm whale myoglobin, at neutral pH and in relation only to the water content. The degree of irreversible transitions can be determined by this technique and is discussed in relation to the solubility of the heat-treated protein samples.

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EXPERIMENTAL

Heat treatment

Thermograms were recorded with a Perkin-Elmer DSC-2 for solid samples and solutions of myoglobin from whale skeletal muscle (salt-free, lyophilized; Sigma Chemical Co.) as a function of water content. The thermograms are characterized by a gradual increase in heat capacity, developing into a peak of intensive heat absorption (Fig. 1),

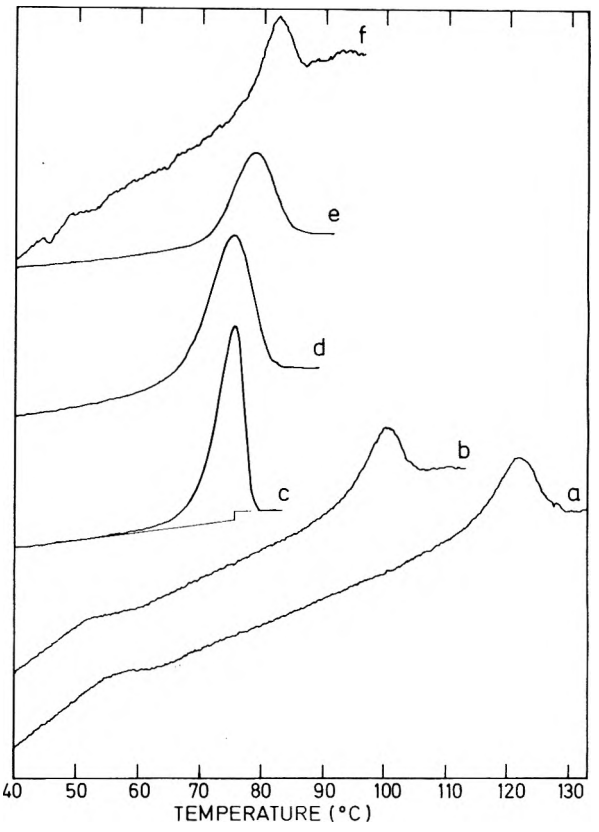

Fig. 1—Temperature dependence of the heat capacity of humidified and wetted samples of myoglobin and myoglobin solutions. Heating rate 10°/min. Curve c illustrates extrapolations of the base line.

Curve	Water content (%)	Instrument sensitivity (mcal/sec)	Sample dry wt (mg)
a	2.8	0.2	2.14
b	7.9	0.2	1.46
c	30.1	0.5	1.91
d	52.9	0.5	2.28
e	74.0	2.0	4.68
f	99.2	0.1	0.14

which is directly associated with the thermal transition of the protein (Jackson and Brandts, 1970; Privalov et al., 1971; Privalov and Khechinashvili, 1974). The thermograms were copied on a paper of constant and known weight per area unit. The apparent transition heat, $\Delta H'_{app}$, was determined by cutting out the peak area and weighing it. The peak area was taken as the area limited by the thermogram and a vertical line connecting the linear extrapolation of the baseline before and after the heat transition at the temperature of the peak maximum (Fig. 1,c). In order to achieve highest reproducibility the temperature of the peak maximum was taken as the transition temperature, T'_m (Privalov and Khechinashvili, 1974).

All DSC-scans were made at a heating rate of 10°C · min^{-1}. Instrument sensitivities of 2, 1, 0.5, 0.2 or 0.1 mcal · sec^{-1} were used. Sample dry weight and water content were determined after the scan by puncturing the sample pan, drying it in a thermostated oven at 105°C for 24 hr and reweighing the sample pan. The sample pans were weighed with an accuracy of 0.01 mg. The scanning range was 25–140°C. Duplicate samples were always analyzed and the apparent transition heat (ΔH_{app}) was determined within ± 0.25 mcal/mg and the transition temperature (T_m) within ± 0.5°C.

In the range 3–30% water content, humidified solid samples were prepared by placing the dry protein in preweighed aluminium volatile-sample pans and equilibrating to relative humidities (RH) up to 97% in desiccators containing saturated salt solutions (Robinson and Stokes, 1959; Hägerdal and Löfqvist, 1973). The sample pans were sealed and reweighed.

In the range 40–75% water content, wetted solid samples were prepared by adding water from a syringe to preweighed sample pans holding a known amount of protein. The sample pans were sealed, reweighed and allowed to equilibrate for 24 hr before the scan was made. Empty sample pans were used as references in the range 3–75% water content. In this range the sample size was 1–3 mg.

In the range 75–99.5% water content, solutions of myoglobin, holding 0.5–25% (w/w) protein, were prepared. The preweighed sample pans were each supplied with 18 µl of myoglobin solution, sealed and reweighed. Sample pans containing 18 µl glass-distilled water were used as references.

T''_m and $\Delta H''_{app}$ of pre-heated myoglobin

In order to analyze the effect of the calorimetric heat treatment on the myoglobin samples at various water contents, larger myoglobin samples (100 mg) were equilibrated to various water contents in small (7 ml) test tubes. The tubes were then sealed and heated in an oil bath, using the same linear heating rate as in the DSC-scan (10°C/min) to a temperature above the upper end of the corresponding DSC-peak, thus ensuring complete heat transition. Finally, these preheated samples were wetted to 75% water content and the new transition temperature (T''_m) and the new apparent transition heat ($\Delta H''_{app}$) were recorded by DSC as described above.

Solubility of preheating samples

The pre-heated samples were also analyzed for solubility. Water suspensions of the samples, holding about 2 mg/ml and 4 mg/ml, were prepared and gently shaken for 24 hr. The final pH varied between 5.8 and 6.8. Corresponding suspensions of untreated myoglobin had a pH of 6.2. The suspensions were centrifuged at 40.000 × G. The concentration of the supernatant was determined by measuring the absorbance at 280 nm ($\epsilon^{0.1\%}_{280}$ = 1.86) of an aliquot after standing for 24 hr in 1M NaOH. The solubility was expressed as a percentage of the original concentration of the suspension.

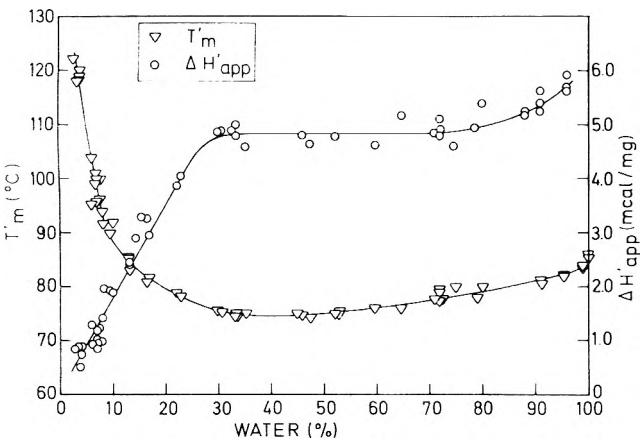

Fig. 2—Transition temperature (T'_m) (▽) and apparent transition heat ($\Delta H'_{app}$) (○) for myoglobin as a function of water content. Heating rate 10°/min. Each symbol represents the measured value for one sample.

RESULTS

T'_m and $\Delta H'_{app}$ of the heat treatment

Figure 1 shows the temperature dependence of the heat capacities of humidified and wetted solid samples, as well as of solutions of myoglobin. The humidified samples are represented by three thermograms, at 3%, 7.5% and 32% water content respectively; the wetted samples by one at 50%, and the solutions by two at 75% and 96% water content respectively.

The variation in T'_m with water content is shown in Figure 2. The curve is characterized by a broad minimum, with a minimum transition temperature of 74°C at about 50% water content. There is also a remarkable increase in T'_m for decreasing water content. The highest T'_m found was 122°C at a water content of 3%. No transition peak was observed for water contents lower than 3%, at maximum sensitivity of the instrument. T'_m also increased again from the minimum with increasing dilution, to 86°C for a water content of 99.6%. Further dilution was limited by the sensitivity of the instrument.

Figure 2 also shows the apparent transition heat ($\Delta H'_{app}$) absorbed by the protein sample during the transition process. This curve is characterized by a steep linear increase in $\Delta H'_{app}$

Table 1—Transition temperature, apparent transition heat and solubility for myoglobin preheated at different water contents[a]

Preheating water content/final temp (°C)	DSC data from Figure 2 for samples of stated water content		Analysis of preheated samples				
			DSC data for samples wetted to 75% water			% solubility	
	T'_m (°C)	$\Delta H'_{app}$ (mcal/mg)	T''_m (°C)	$\Delta H''_{app}$ (mcal/mg)	$\Delta H'_{app} + \Delta H''_{app}$ (mcal/mg)	2 mg sample/ml	4 mg sample/ml
2.3%/130°	122°	0.4	77.2	4.0–4.2	4.5	88	83
9.5%/110°	89°	1.6	77.6	3.8–3.9	5.4	79	86
15.6%/90°	82°	2.7	78.1	2.2–2.7	5.1	48	50
20.6%/90°	79°	3.6	79.8	1.4	5.0	26	–
35.2%/85°	75°	4.7	79.8	0–0.1	4.8	4	2

[a] Heating rate 10°/min; duplicate samples. Maximum error in transition temperature ± 0.5°C: in apparent transition heat ± 0.25 mcal/mg; in solubility ± 5%.

from 0.5 to 4.8 mcal/mg in the interval 3–30% water content. For water contents > 30% there is a plateau followed by a slow increase in the apparent transition heat from 4.8 mcal/mg to 5.8 mcal/mg at 96% water. At water contents higher than 96% the determination of $\Delta H'_{app}$ becomes too uncertain to allow quantitative evaluations. A second scan, repeated after 15 min of cooling, gave no transition peak for any of the samples.

T''_m, $\Delta H''_{app}$ and solubility of preheated samples

The effect of the heat treatment at five different water contents (2.3, 9.5, 15.6, 20.6 and 35.2% water) was investigated. The solubility of the preheated samples was measured and after wetting to 75% water, the transition temperature (T''_m) and the apparent transition heat ($\Delta H''_{app}$) were also measured. The data are summarized in Table 1. It was found that T''_m for these samples was 78.5°C ± 1.2°C, which, within experimental error, is the same as T'_m found for untreated myoglobin at 75% water content (Fig. 2). A heat absorption peak, which was hardly significant, was found for the sample holding 35.2% water during the heat treatment. As seen in Figure 2 the steep increase in $\Delta H'_{app}$ is complete at this water content. Adding $\Delta H'_{app}$ of the heat treatment and $\Delta H''_{app}$ after the heat treatment for the five analyzed samples gives a constant value of 5.0 ± 0.3 mcal/mg. Within experimental error this is the same value as was found for untreated myoglobin wetted to 75% water content (Fig. 2). It was also found that the solubility of the heat-treated samples, at different water contents, was independent of the original concentration of the water suspension. Furthermore, there was a linear relationship between the solubility and $\Delta H''_{app}$ after heat treatment, as shown in Figure 3.

Heating rate

The effect of the heating rate on the transition temperature (T'_m) and the apparent transition heat ($\Delta H'_{app}$) was investigated for duplicate myoglobin samples equilibrated to 97% RH, i.e., holding about 32% water. The experimental data are collected in Table 2. When the heating rate is increased by about 20 times (from 1.25 to 20°C/min), T'_m is increased by about 10°C. However, the starting temperature of the heat transition remains constant within ± 1°C. $\Delta H'_{app}$ also shows a considerable increase from 3.6 mcal/mg to 5.2 mcal/mg.

DISCUSSION

Effect of heating rate on T'_m and $\Delta H'_{app}$

As the heat transition peak is a result of thermodynamic and kinetic phenomena, T'_m is shifted further upwards for kinetic reasons, when the heating rate is increased (Table 2). The increased T'_m was not due to heat resistance in either the protein sample or the calorimeter, as the onset of the heat transition remained constant within ± 1°, which was also found by Donovan and Ross (1973).

Similarly, increasing the heating rate 20 times resulted in an increase in $\Delta H''_{app}$ from 3.6 to 5.2 mcal/mg (Table 2). Incidently, Donovan and Ross (1973) found the same enthalphy of denaturation when using heating rates differing by a factor of five in their study of the avidin-biotin complex. The values given in Table 2 rest on duplicate scans with a maximum error of ± 0.2 mcal/mg. Furthermore, there is at no point a deviation from the tendency of an increase in $\Delta H'_{app}$ with increasing heating rate. The divergencies in results might be due to great differences in protein concentration: 70% in this investigation and 7% in the investigation of Donovan and Ross (1973). The found increase in $\Delta H'_{app}$ with increasing heating rate might imply that different kinetic procedures give rise to different final states of the protein samples at high protein concentrations.

Finally, it should be noted that a slow scanning rate was not recommended (Goldberg and Prosen, 1972) when using differential scanning calorimetry to make quantitative evaluations of protein transitions, which occur over a wide temperature range.

Effect of low water content (< 30%) on T'_m and $\Delta H'_{app}$

The apparent transition heat ($\Delta H'_{app}$) for myoglobin increases linearly up to 4.8 mcal/mg with increasing water content up to 30% (Fig. 2). In fact, the linear relation between $\Delta H'_{app}$ and water content on a dry matter basis was more complete than between $\Delta H'_{app}$ and water content on a wet matter basis (Fig. 2), as is used elsewhere in this communication. This is to be expected, since $\Delta H'_{app}$ was also calculated on a dry matter basis.

Recently, Luescher et al. (1974) and Rüegg et al. (1975) reported a similar increase in transition heat with increasing water content for tropocollagen and β-laktoglobulin, respectively. These results were interpreted as an increase in hydrogen bonds in the protein sample as the water content was increased up to 30%.

Table 1 shows, however, that the samples with the lowest $\Delta H'_{app}$ had the highest solubility and $\Delta H''_{app}$ after the heat treatment. Thus, removing water from myoglobin at water

Table 2–Transition temperature and apparent transition heat for myoglobin at different heating rates[a]

Heating rate °/min	T'_m (°C)	$\Delta H'_{app}$ (mcal/mg)
1.25	68.9	3.6
2.5	70.4	4.1
5	72.9	4.3
10	75.4	4.9
20	78.5	5.2

[a] Water content 32%, duplicate samples, Maximum error in transition temperature ± 0.5°C; in apparent transition heat ± 0.2 mcal/mg.

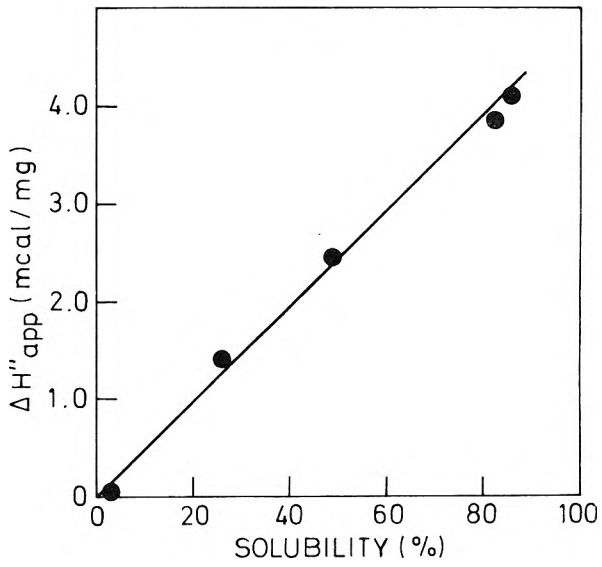

Fig. 3–$\Delta H''_{app}$ of preheated myoglobin after wetting to 75% water content as a function of solubility. The symbols represent the mean value of duplicate samples with an error of ± 0.25 mcal/mg in apparent transition heat and ± 5% in solubility.

contents lower than 30%, leads to a decreasing amount of the protein sample being irreversibly transformed, as measured by the solubility and $\Delta H''_{app}$ after wetting to 75% water content.

Similarly, Bull and Breese (1968) found that there was an increase in solubility for ovalbumin when heat treated at decreasing water contents lower than 30%. These authors put forward the hypothesis that during successive water removal from the protein, inter- and intra-molecular electrostatic interactions and hydrogen bonds have to be established in order to satisfy the sites earlier occupied by water. These inter- and intra-molecular interactions should be the reason for the increased thermostability of the proteins.

On the other hand, Donovan and Beardslee (1975) found that trypsin could be stabilized against thermal transitions, in dilute solutions, by interaction with either soybean trypsin inhibitor or ovomucoid. As these specific protein-protein interactions are established in competition with an excess of water molecules, they represent much stronger molecular interactions than can be established due to the removal of water. The increased thermostability found for the complex of trypsin-soybean trypsin inhibitor and trypsin-ovomucoid respectively, was manifested only in an increased transition temperature, whereas ΔH_{app} for the complex was the sum of the ΔH_{app} for the separate proteins.

It is therefore our opinion that the decrease in irreversible thermal transitions at water contents below 30% found in this investigation is mainly due to the lack of a water medium bringing about thermal transitions (Lewin, 1974). During the course of the heat treatment the present water molecules presumably concentrate to a fraction of the protein sample, which undergoes irreversible transition, whereas the rest of the sample remains in its original conformation, due to the lack of a water medium. This hypothesis assumes, however, that the catalytic effect of water (Chirgadze and Ovsepyan, 1972) only works for longer periods of time (24 hr) than used in the DSC scan.

The increased protection against irreversible thermal transition induced by water contents of lower than 30% was accompanied by a steep increase in T'_m. Similarly, Barker (1933) showed that the denaturation temperature for egg albumin, as determined from the degree of solubility, was a linearly decreasing function of the relative humidity. As water molecules absorbed by proteins become more tightly absorbed at decreasing relative humidities (Kuntz and Kauzmann, 1974), the increased T'_m reflects the increased amount of energy needed to mobilize these water molecules.

Effect of medium water content (30–80%) on T'_m and $\Delta H'_{app}$

In this region the transition temperature and apparent transition heat are mainly constant (Fig. 2). Samples preheated in this region of water content gave no further peak of heat transition upon increased wetting. Therefore, the myoglobin samples underwent complete and irreversible heat transition at these water contents.

Effect of high water content (> 80%) on T'_m and $\Delta H'_{app}$

Also in this region it was found that the entire myoglobin sample underwent irreversible heat transition. There is, however, an increase in transition temperature as well as in apparent transition heat (Fig. 2).

The increase in transition temperature points out that the protein is stabilized against heat transition upon dilution. It has been shown (Brunori et al., 1972) that already 5% ethanol lowered the transition temperature from 63° to 56°C for *Aplysia* myoglobin. Similarly, as low protein concentrations one protein molecule might be regarded as acting as an apolar solvent of the other.

Privalov et al. (1971) investigated the reversible thermal transition of myoglobin in dilute solutions (< 0.3%) at pH > 9.5. The heat of thermal denaturation, in dilute solutions at neutral pH, was estimated to 8.5 mcal/mg. These authors also pointed out that at higher protein concentrations (> 0.3%) exothermic aggregation over-shadows some of the endothermic transition enthalpy. Therefore, the increase in $\Delta H'_{app}$ at water contents above 80% implies that aggregation becomes less intense as the water content is increased, i.e., as the protein concentration is decreased.

Heat treatment as an all-or-none process?

Table 1 shows $\Delta H'_{app}$ for myoglobin samples holding different amounts of water below 35.2%, $\Delta H''_{app}$ for these samples after wetting to 75% water and the solubility of the samples. Firstly, the sum of $\Delta H'_{app}$ and $\Delta H''_{app}$ was, within experimental error, a constant value of 5.0 mcal/mg. This was the same value as was found for untreated myoglobin having a water content of about 75%. Secondly, there was a linear relation between the solubility and $\Delta H''_{app}$, as shown in Figure 3. Thirdly, the solubility was the same for water suspensions having concentrations of both 2 and 4 mg/ml. In certain experiments 10 times higher concentrations were used, and the solubility as a percentage was, within experimental error, still the same. Finally, the heat-treated samples after wetting to 75% water content all had the same T''_m, 78.5 ± 1.5°C (Table 1), which, within experimental error, is the same as for untreated myoglobin at this water content.

These facts support the hypothesis that at water contents below 30% only a certain fraction of the protein sample undergoes irreversible transition and the rest remains native or undergoes reversible transition. When wetting these heat-treated samples to 75% water content and performing a DSC-scan, the remaining native or reversibly transformed fraction also undergoes irreversible thermal transitions. Similarly, when the heat-treated samples are suspended in water, the corresponding fraction of the sample becomes soluble (Fig. 3).

These findings could be interpreted in terms of the so-called two-state transition theory (Lumry et al., 1966), which has been developed for completely reversible systems on a thermodynamic basis (i.e., in dilute solutions). The main argument in this theory is that all states between the native and denatured state of a protein molecule are energetically unstable and do not exist for a measurable period of time. It has been widely discussed; for example, McKenzie and Ralston (1971) have reviewed the limited applicability of the two-state theory to different reversible protein transition processes.

All the systems discussed in the present investigation, however, have protein concentrations giving rise to irreversible aggregation when heat treated. Thus, the two-state transition theory is not applicable. These aggregates might consist of native as well as denatured molecules (Tanford, 1969). Therefore, the fraction of the protein sample which remains insoluble and accounts for the difference between $\Delta H''_{app}$ and $\Delta H'_{app}$ at 75% water content might represent a variety of molecular conformations. Similarly, the soluble fraction, accounting for $\Delta H''_{app}$ after wetting to 75% water content, might consist of native molecules and molecules which have undergone minor as well as reversible transitions. On the basis of the results in the present investigation, it is therefore not possible to determine whether the thermal denaturation of myoglobin at low water content is an all-or-none process or a gradual denaturation of each molecule.

Applications

The greatly increased protection against irreversible thermal transitions due to the removal of water below 30% shown in Figure 2 is of the utmost importance in all drying procedures involving biological material. In a separate experiment it was found to be possible to heat a myoglobin sample holding 8% water at a rate of 10°/min to 90°C without causing any thermal denaturation as shown by both the $\Delta H''_{app}$ after wetting to 75% water content and the solubility of the sample.

As a matter of fact, in the production of protein isolates

from rape seeds (Appelqvist and Josefsson, 1967; Gillberg and Törnell), rape seeds holding 8% water are heated to 90°C in order to inactivate the enzyme myrosinase, which otherwise gives rise to toxic products. This heat treatment does not result in any deterioration of the functional properties of the protein isolate. The selective influence of the heat treatment on the enzyme myrosinase at this particular water content might be due either to the enzyme having a poor thermostability compared to the rest of the protein material, or the fact that a very small conformational change may completely destroy the enzyme's activity.

The DSC-technique used in this investigation turned out to be a valuable analytical tool for characterizing thermal transitions in proteins, in solution as well as for solid samples. This technique can also differentiate between the thermal effect as discussed in this paper and the kinetic effect. It also permits a simple and quick analysis of the effect of a thermal denaturation compared to the solubility analysis, which was shown by the correspondence of these two parameters (Fig. 3). The small amount of sample required, 1–3 mg, for each DSC-scan is especially valuable when working with model proteins, as in the present investigation.

The correspondence between the solubility and the $\Delta H''_{app}$ also points out a method for estimating the degree of irreversible denatured material in e.g., protein concentrates and isolates. From work in progress it has been found that fish, meat and cereals give similar peaks of thermal transition as found in the present investigation. This has also been shown by Karmas and DiMarco (1969) for beef muscle tissue. The DSC-technique might therefore be a valuable tool for following the degree of irreversible denaturation caused by different steps in the production of protein concentrates and isolates, as well as in food processing.

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Ms received 10/28/75; revised 1/16/76; accepted 1/20/76.

This investigation was supported by a research grant from the Swedish Board for Technical Development.

We express our gratitude to Bo Löfgvist and Ingemar Wadsö for many valuable discussions, to Margret Månsson for introducing us to differential scanning calorimetry, and to Ingun Sunden-Cullberg for skillful technical assistance.

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DISCOLORATION OF CHILLED SWEET POTATO [Ipomoea batatas (L.) Lam.] ROOTS: FACTORS RELATED TO CULTIVAR DIFFERENCES

ABSTRACT

Roots of sweet potato cultivar Jewel, and two unnamed selections (213 × 228-1, and 257 × 255-8) were stored at 5°C or 14°C for 10 wk. Increased discoloration of certain cultivars of chilled sweet potato roots is apparently not due to an increase in the amount of extractable peroxidase or chlorogenic acid oxidase activity. Chilled tissues of 213 × 228-1 discolored and showed an increase in pH and chlorogenic acid. Chilled tissues from roots of 257 × 255-8 exhibited an increase in pH, but did not increase in chlorogenic acid content and were only slightly discolored. Tissues of Jewel changed little in pH or chlorogenic acid content and discolored the least of the three cultivars. Chlorogenic acid discolored under the influence of high pH. The data indicate that differences among cultivars in discoloration resulting from chilling injury may be due to differences in tissue pH and chlorogenic acid content.

INTRODUCTION

DISCOLORATION of a commodity can seriously detract from consumer acceptance. Tissue discoloration is a typical symptom of chilling injury. Some sweet potato cultivars (cvs.) discolor more than others after chilling (Cooley et al., 1954; Kushman and Pope, 1973; Lewis and Morris, 1956; Lieberman et al., 1958). Other commodities such as apples (Lyons, 1973); yams (Coursey, 1968); peaches (Lyons, 1973); pineapples (Miller and Heilman, 1952); eggplant (McColloch, 1966); snapbeans (Watada and Morris, 1966a, b); plums (Lyons, 1973); bananas (Murata, 1969; Haard and Timbie, 1973); muskmelon rinds (Ramsey and Smith, 1961); pepper seeds (Kozukue and Ogata, 1972); and pepper calyx (Lacy and McColloch, 1962) also discolor during exposure to chilling temperatures.

Discolored products can result from oxidized phenols. Various phenols are known to increase during chilling. Chilled bananas exhibit an increased content of dopa (3,4-dihydroxyphenylalanine) and tyrosine (Murata, 1969). Chlorogenic acid has been found to increase in concentration in chilled sweet potato roots (Lieberman et al., 1958, 1959; Marr, 1971) and pepper seeds (Kozukue and Ogata, 1972). The activity of the polyphenol oxidase enzyme which should act on these substrates to form discolored products has not been investigated in chilled tissues. Peroxidase can discolor polyphenols. Peroxidase increases in chilled bananas (Haard and Timbie, 1973).

This study was designed to investigate factors which may cause the discoloration response of the roots of three sweet potato cvs. to a chilling temperature. The activity of peroxidase, chlorogenic acid oxidase, and the chlorogenic acid content of the periderm and parenchyma tissues were monitored over a 10-wk period to see how they related to discoloration. The pH which could have significant effects on metabolism was measured as this changes in response to chilling (Kushman and Pope, 1970).

MATERIALS & METHODS

SWEET POTATO ROOTS of the cv. Jewel, and two advanced selections 213 × 228-1 and 257 × 255-8 were supplied by Dr. D.T. Pope from the North Carolina Sweet Potato Breeding Program. These cvs. were used because they had shown a wide range in response to chilling injury-induced discoloration in previous studies (Kushman and Pope, 1973). Jewel discolored slightly during chilling, 213 × 228-1 discolored severely, and 257 × 255-8 was intermediate in discoloration. The roots were cured for 7 days at 29.4°C, 90–95% relative humidity, and then stored at 14°C and 90% relative humidity until used. The design of the experiment included one root per replicate with four replicates per treatment. Roots were examined after storage at 5°C or 14°C for 0, 2, 4, 6, 8, or 10 wk.

Enzyme extraction

Two areas of each root were sampled. The periderm tissue was separated at the cambium and included the unsterilized skin. The central parenchyma tissue was taken from the center of the roots using a 12 mm cork borer. The extraction technique was a modified version of that of Matsuno and Uritani (1972). The enzyme was extracted from 5g of tissue in 20 ml of cold 0.05M sodium phosphate buffer (pH 7.0) for 5 min in a VirTis blender operated at medium speed. The resulting mixture was centrifuged at 27,000 × G for 15 min at 4°C. The filtered supernatant fluid was used in enzyme assays.

Peroxidase assay

The peroxidase assay used was a modified version of the assay of Matsuno and Uritani (1972). Hydrogen peroxide was added last to start the reaction. The progress of the reaction at 22°C was followed at 450 nm over a 3-min period. Peroxidase activity was calculated from the change in optical density during the last 2 min of the reaction.

Chlorogenic acid oxidase assay

A spectrophotometric assay technique was developed for the measurement of chlorogenic acid oxidase activity. The reaction mixture consisted of 200 µl of 25 mM chlorogenic acid, 5–320 µl of enzyme extract (depending on the cv. and amount of chilling), and enough 0.1M sodium phosphate buffer (pH 6.0) as used by Hyodo and Uritani (1965, 1966) in order to bring the total volume to 1 ml. The reactions were carried out in a spectrophotometer cuvette. Chlorogenic acid was added last to initiate the reaction. The reaction rate was monitored over a 6 min period at 400 nm. Chlorogenic acid oxidase activity was calculated from the increase in optical density during the last 5 min of the reaction.

In all enzyme assays it was verified that the amount of product formed was proportional to the concentration of enzyme in the reaction by repeating each assay with two concentrations of enzyme.

pH determination

The pH of each tissue was determined with a pH meter using a slurry prepared from a 10- to 20-g sample of tissue blended in two volumes of distilled water for 5 min.

Chlorogenic acid content determination

Five-g samples of tissue were extracted with 30-ml of 50% ethanol for 5 min in a VirTis blender (Lieberman et al., 1959). The mixture was centrifuged at 27,000 × G for 15 min and the supernatant fluid removed, the volume measured, then filtered through Whatman No. 2 filter paper. Chlorogenic acid content was determined by reading the optical density of the extract at 325 nm, the absorption maximum for chlorogenic acid, (Merck Index, 1968). These values were compared to a standard curve of chlorogenic acid in 50% ethanol.

Nonenzymatic discoloration of chlorogenic acid

To determine the effect of pH on discoloration, chlorogenic acid solutions of 0.5, 1.0, 2.0 and 4.0 mM were incubated with 0.1M sodium phosphate buffer over a 5.4–7.4 pH range at 0.2 intervals. The solutions were incubated for 1 hr at 22°C before reading the optical density at 410 nm. At this wavelength, chlorogenic acid in acidic solution

absorbed little light, but under alkaline conditions a brown color developed which absorbed light at 410 nm.

To determine the effect of pH on the discoloration of a sweet potato extract, aqueous extracts from periderm and parenchyma tissues of 213 × 228-1 were incubated for 1 hr with a series of 0.1M sodium phosphate buffers over a pH range 5.4–7.4 at 0.4 unit intervals. The optical densities of the extracts were read at 410 nm.

RESULTS & DISCUSSION

STORAGE at the nonchilling temperature produced little or no discoloration as observed visually. Storage at 5°C caused discoloration which increased with time in all tissues of all the cvs. The periderm tissue discolored more than the parenchyma tissue. After 6–10 wk of chilling, it was noted that the vascular tissues had turned black. This histological change has also been reported to occur in chilled banana peel tissue (Murata, 1969). The cv. Jewel discolored much less than the other cvs. The cv. 213 × 228-1 discolored severely during storage at 5°C. The difference in response among cvs. confirmed observations by Kushman and Pope (1973).

Peroxidase activity

Storage at 5°C did not affect peroxidase activity (Fig. 1). The peroxidase activity of the periderm tissue of Jewel was highest and was less in 213 × 228-1 and least in 257 × 255-8. The order of peroxidase activity in the parenchyma tissue was Jewel, 257 × 255-8, and 213 × 228-1. Peroxidase has been implicated in the discoloration of Irish potatoes (Weaver et al., 1971) and apples (Lyons, 1973). The sweet potato cv., Jewel, which had the highest peroxidase activity in this study, discolored the least during chilling. The progressive discoloration which occurred in sweet potato roots stored at 5°C cannot be attributed to a progressive increase in the amount of peroxidase present in the roots. Haard and Timbie (1973) observed severe peel blackening in bananas stored at 5°C which coincided with the initiation of a rise in soluble peroxidase.

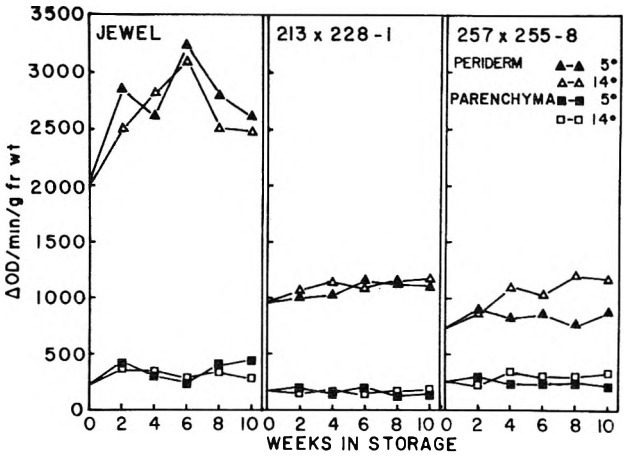

Fig. 1–The effect of temperature and time in storage on peroxidase activity in the periderm and parenchyma tissues of three sweet potato cultivars.

Chlorogenic acid oxidase activity

Chlorogenic acid oxidase activity tended to be lower at 5°C than at 14°C (Fig. 2). Within each cv., the chlorogenic acid oxidase activity was higher in the periderm tissue than in the parenchyma tissue. 213 × 228-1 had the highest activity in both the periderm and parenchyma tissues. It was also the selection that showed the greatest degree of discoloration when chilled.

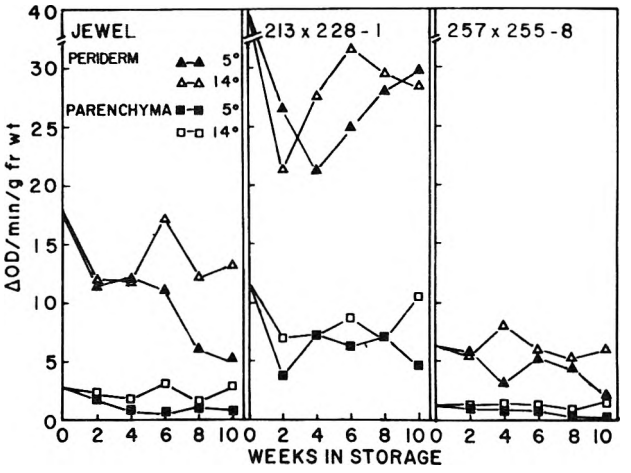

Fig. 2–The effect of temperature and time in storage on chlorogenic acid oxidase activity in the periderm and parenchyma tissues of three sweet potato cultivars.

Chlorogenic acid content

Jewel and 257 × 255-8 showed little or no increase in chlorogenic acid content in either tissue during storage at 5°C (Fig. 3). On the contrary, both tissues of 213 × 228-1 increased three- to fourfold in chlorogenic acid content as a result of storage at 5°C. The chlorogenic acid content was higher in the periderm than parenchyma tissue. The roots stored at 14°C maintained a relatively constant level of chlorogenic acid.

Lieberman et al. (1958, 1959) reported that the chlorogenic acid content of sweet potato roots increased during chilling, and that it may be related to discoloration. However, Minamikawa et al. (1961) failed to detect any increase in chlorogenic acid content due to chilling storage. Differences in varietal responses in chlorogenic acid content during chilling may account for the contradictory reports.

pH

The pH of both tissues of 213 × 228-1 and 257 × 255-8 increased rapidly as the time at 5°C increased (Fig. 4). In the case of Jewel, the pH decreased during the first 4–6 wk of chilling, then began to increase. The pH of the nonchilled (14°C) tissues generally decreased during storage with some showing an increase near the end of the test period. It was found that periderm tissue was more acidic than parenchyma tissue. Any pH changes that occurred during storage occurred

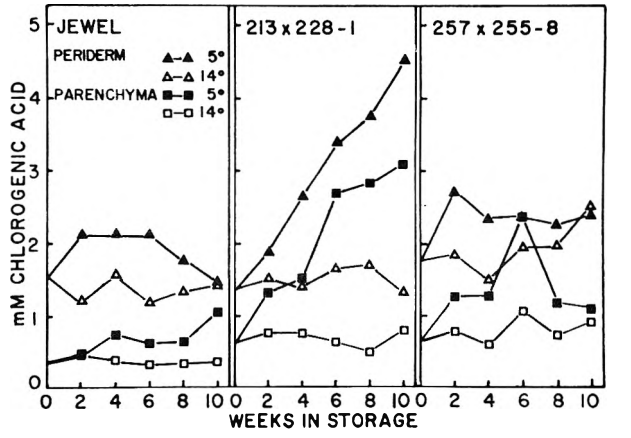

Fig. 3–The effect of temperature and time in storage on the chlorogenic acid content in the periderm and parenchyma tissues of three sweet potato cultivars.

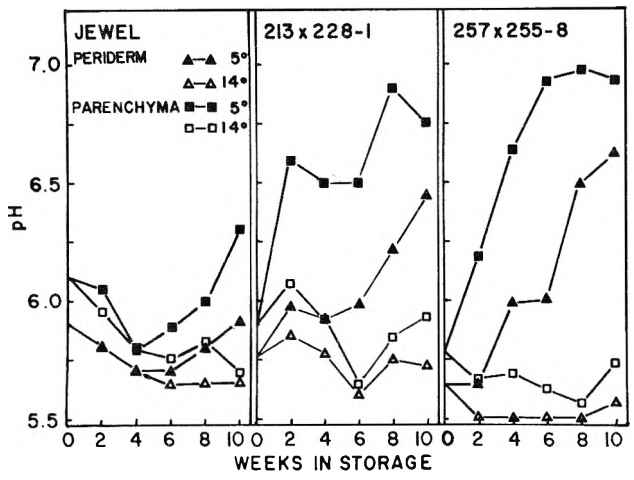

Fig. 4—The effect of temperature and time in storage on the pH in the periderm and parenchyma tissues of three sweet potato cultivars.

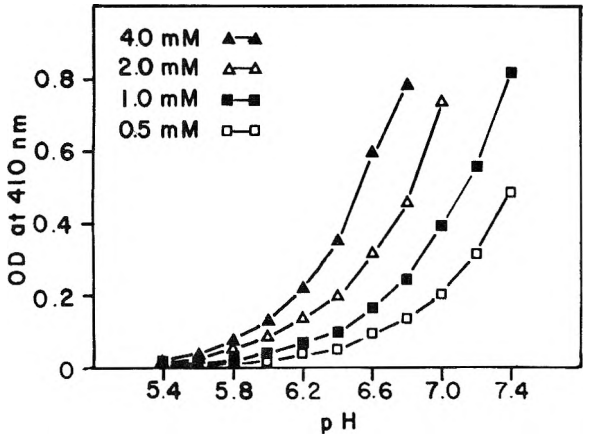

Fig. 5—The effect of pH and concentration on the nonenzymatic discoloration of chlorogenic acid.

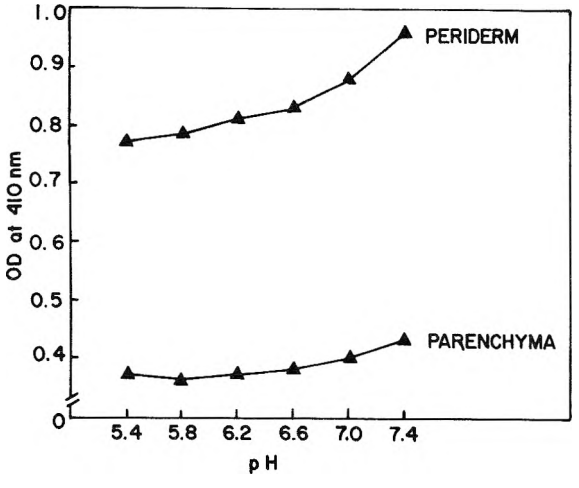

Fig. 6—The effect of pH on the discoloration of an aqueous extract of sweet potato root tissues, cultivar 213 x 228-1.

concomitantly in both tissues. Kushman and Hoover (1965) and Kushman and Pope (1973) reported that the pH of the root tissue increased with time at a chilling temperature after an initial decrease. The rate of change of the pH increased as the temperature decreased. Kushman and Pope (1973) found that the pH of some varieties responded more rapidly to chilling than other varieties. The differences in pH of the periderm and parenchyma tissues have never been reported.

Nonenzymatic discoloration of chlorogenic acid

The potential effect of pH changes in causing discoloration were recognized when it was found that chlorogenic acid discolored in response to an increase in pH (Fig. 5). This effect of pH on the discoloration of chlorogenic acid had been reported for pH values which were very alkaline (Sondheimer, 1962). Under the influence of alkaline pH, phenols dissociate to form phenoxide ions. This ionization in turn produces a shift in the ultraviolet absorption maximum toward longer wavelengths (Seikel, 1964). The pH values and chlorogenic acid concentrations chosen for this study encompassed those found in chilled and nonchilled sweet potato roots. The brown discoloration measured at 410 nm occurred instantly upon pipetting the chlorogenic acid into the buffer. Also, the brown discoloration could be reversed by lowering the pH of the solution. These observations are consistent with the idea that discoloration induced by pH is the result of an ionization phenomenon.

Data in Figure 6 demonstrate that pH has an effect on the discoloration of aqueous extracts made from two different tissues of nonchilled roots of the selection 213 x 228-1. Because the pigments normally present in the extract absorb significantly at 410 nm, it was necessary to dilute the extracts considerably to perform the experiment. The chlorogenic acid contents of the extracts from the periderm and parenchyma were 0.33 mM and 0.11 mM respectively, which was 10% of that in the tissue. As could be predicted from the data in Figure 5 and the chlorogenic acid concentrations of the extracts, a much greater increase in optical density was obtained by adjusting the pH of the periderm extract from pH 5.4 to 7.4 than by similar adjustment in the pH of the parenchyma extract. Sistrunk (1971) canned sweet potatoes in solutions buffered at various pH values. He found that color was improved as indicated by visual color ratings and higher 'L' values as measured by the Color Difference Meter as the pH was decreased from 8.0 to 3.0.

In summary, the results of this study indicate that storage at a chilling temperature of 5°C had no significant effect on the amount of peroxidase or chlorogenic acid oxidase enzymes in tissues of the roots of the three sweet potato cvs. tested. Because there was no change in the total content of these enzymes during chilling, it was concluded that an increase in the amount of either enzyme could not be responsible for the increased discoloration observed at 5°C.

It is possible that discoloration was mediated by chlorogenic acid oxidase and/or by peroxidase due to changes in compartmentalization which allowed greater contact between enzymes and substrates. Changes in compartmentalization are known to occur in chilled tissues (Lyons, 1973). Increased polyphenol concentration might have caused greater discoloration due to enzyme substrate saturation phenomena. Changes in tissue pH could have altered enzyme activity to cause increased discoloration.

While these possibilities exist, it was found that chlorogenic acid discolored in response to increased pH. The amount of discolored product was greater at any given pH when the chlorogenic acid concentration was greater. These findings correlate well with visual observations of sweet potato roots which may explain differences between cvs. in discoloration. In the selection 213 X 228-1 which discolored severely during chilling injury, the tissues increased in both pH and chlorogenic acid content. The selection 257 X 255-8 which was inter-

mediate in its discoloration response during chilling, exhibited an increase in pH but no increase in chlorogenic acid concentration. Jewel, the cv. which discolored the least, increased little in either pH or chlorogenic acid content during chilling injury. Storage at 14°C did not induce discoloration and was associated with stable pH and chlorogenic acid content. Thus, discoloration may be mediated in part by ionization of chlorogenic acid at high pH values. A greater comprehension of factors which are responsible for changes in pH and chlorogenic acid concentration should ultimately provide greater clarification of differences in responses of sweet potato cvs. to chilling injury.

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Ms received 9/6/75; revised 10/28/75; accepted 11/5/75.

Journal Article No. 4761 of the North Carolina Agricultural Experiment Station. The use of trade names in this publication does not imply endorsement by the North Carolina Experiment Station of products named, nor criticism of similar ones not mentioned.

This work was conducted in part to fulfill the requirements of W.C. Porter for a Ph.D. degree.

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IRRADIATED FOOD: VALIDITY OF EXTRAPOLATING WHOLESOMENESS DATA

ABSTRACT

Criteria are considered for validly extrapolating the conclusions reached on the wholesomeness of an irradiated food receiving high doses to the same food receiving a lower dose. A consideration first is made of the possible chemical mechanisms that could give rise to different functional dependences of radiolytic products on dose. It is shown that such products should increase linearly with dose and the ratio of products should be constant throughout the dose range considered. The assumption, generally accepted in pharmacology, then is made that if any adverse effects related to the food are discerned in the test animals, then the intensity of these effects would increase with the concentration of radiolytic products in the food. Lastly, the need to compare data from animal studies with foods irradiated to several doses against chemical evidence obtained over a comparable dose range is considered. It is concluded that if the products depend linearly on dose and if feeding studies indicate no adverse effects, then an extrapolation to lower doses is clearly valid. This approach is illustrated for irradiated codfish. The formation of selected volatile products in samples receiving between 0.1 and 3 Mrads was examined, and their concentrations were found to increase linearly at least up to 1 Mrad. These data were compared with results from animal feeding studies establishing the wholesomeness of codfish and haddock irradiated to 0.2, 0.6 and 2.8 Mrads. It is stated, therefore, that if ocean fish, currently under consideration for onboard processing, were irradiated to 0.1 Mrad, it would be correspondingly wholesome.

INTRODUCTION

THE VALIDITY of extrapolating data on the wholesomeness (i.e., safety and nutritional value) of irradiated food, about which Diehl (1973) has requested information, depends on how the formation of radiolytic products is influenced by absorbed dose. For the ideal case in which the absolute amount of each radiolytic product increases proportionately as the dose is increased, an extrapolation of data from different wholesomeness studies should be valid. For complex cases, specific chemical and wholesomeness evidence from experiments covering a wide range of dose must be examined. Consequently, this paper (a) discusses possible functional dependences of product yield on dose; (b) presents recent experimental results on selected radiolytic products extracted from irradiated codfish; and (c) considers the combined significance of these results and the data from animal feeding studies on irradiated codfish and haddock towards extrapolating the wholesomeness conclusions.

EXPERIMENTAL

SELECTED VOLATILE PRODUCTS were collected from cans of codfish irradiated at 1 ± 1°C to doses of 0.1–3.0 Mrads and then analyzed by gas chromatographic and/or mass spectrometric techniques (GC/MS) (Merritt, 1970). The codfish, maintained between 0° and 5°C was filleted with the skin retained, placed in large cans providing a three-fold excess headspace, sealed in these cans without purging the air, and irradiated, about 30 hr after the catch, with ^{60}Co gamma rays at a dose-rate of 1 Mrad per hour. During the period of approximately 3 days between irradiation and analysis, the samples were stored frozen at −18°C. Hydrogen and carbon dioxide in the headspace were determined from PVT measurements and mass spectrometric (CEC Model 21-103) analyses. Of the volatiles in the fish, those distillable at −80°C and condensable at −140°C were vacuum fractionated and determined by GC/MS analysis. The chromatographic separation was accomplished using a 215m × 0.8 mm capillary column coated with OV-101 containing 5% Igepal Co 880, and the eluates were identified with a CEC Model 21-491 mass spectrometer.

RESULTS & DISCUSSION

Influence of dose on product yields

Because food is a multiphasic and multicomponent system, the treatment of product concentration vs dose has been simplified to make it tractable. It is assumed that food, which is taken to be composed of a major substrate, S, and a minor component, S′, upon exposure to ionizing radiation gives rise to: a major product, M, formed directly and indirectly; an intermediate species, X; and minor products A, B and C, formed directly, and D, E and F, formed through the involvement of X. (The intermediate, X, can be identified with various species formed in the water, protein, or fat fractions upon exposure to ionizing radiation such as $H^{\bullet}$, e_s^-, $OH^{\bullet}$, $CH_3^{\bullet}$, $CH_3C^{\bullet}O$, etc.) The following generalized reaction scheme describes the chemistry:

$$S \text{ in } \xrightarrow{\gamma,\, e^-} M, X, A, B, C \ldots \quad (1)$$

$$S + X \rightarrow M \quad (2)$$

$$B + X \rightarrow D \quad (3)$$

$$S' + X \rightarrow E \quad (4)$$

$$E + X \rightarrow F \quad (5)$$

Embodied in this scheme are a number of different concentration-dose relations. Reactions 1 through 3 account for the concentrations of A and C rising linearly with absorbed dose; for the concentration of B increasing initially but then reaching a steady-state value; and for the concentration of D increasing linearly after the concentration of B becomes constant. Reactions 4 and 5 account for the concentration of E increasing initially, reaching a maximum value, and then decreasing. E continues to be produced as long as component S′ exists in high enough concentration to compete for X; it is eventually lost because destruction through 5 is not compensated by formation through 4 when S′ has been depleted.

Despite its oversimplifications, this model of the chemistry describes the competing processes that give rise to the different concentration-dose relations and in principle enables one (using an approximate mathematical treatment and using representative G-values and rate constants) to estimate the critical dose at which nonlinearity would be observed. (This treatment assumes that X reaches a steady-state concentration, which in the case of $X=H^{\bullet}$ is determined primarily by reaction 2, and leads to expressions for the concentrations of products as a function of dose, which for B, prior to the steady-state being attained is:

$$B = a \cdot b\,(1 - e^{-bD})$$

where a and b are constants and D = dose. G-value is defined as the number of molecules of a product formed per 100 eV of energy absorbed.) Since the composition of food is so complex, most of these possible responses would be expected at very high doses. The actual responses for fish can be determined by analyzing the radiolytic products formed over a wide dose range.

Volatiles from irradiated codfish

The results shown in Figure 1 indicate that the concentrations of many of the radiolytic products increase with increasing absorbed dose. Most of these products are formed also through microbial action (King et al., 1972) as evidenced by their presence in the control. This nonradiolytic contribution to the product yield arises during the time between catching the fish and freezing the irradiated samples. The slopes of the lines in Figure 1 are relatively constant for all products up to about 1 Mrad and for some up to 3 Mrads.

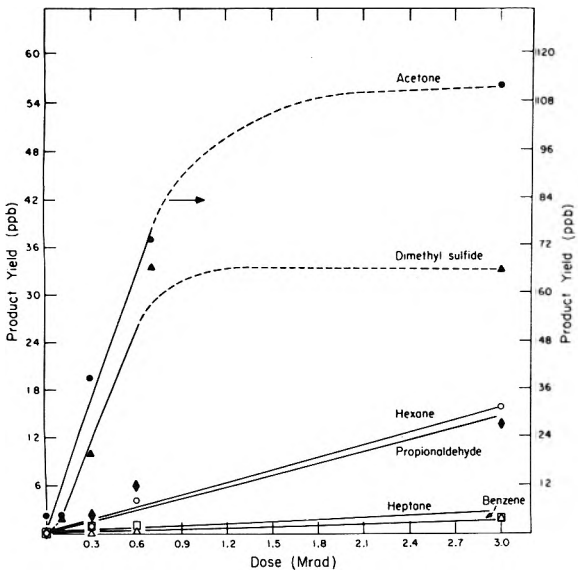

Fig. 1—Yield of volatile products as a function of dose. (Hexane, ○; heptane, □; benzene, △; acetone, ●; dimethyl sulfide, ▲; propionaldehyde, ♦.) All of the yields are given in terms of parts by weight of compound per 10^9 parts by weight of the codfish sample (ppb). The ordinate scale on the left relates to all of the products except acetone, which should be referred to the ordinate scale on the right.

Table 1—Identification and concentrations of representative volatiles extracted from control and irradiated codfish samples

	Concentration (ppb)[a]		
Compound	Dose: 0 Mrad	0.1 Mrad	3.0 Mrad
Acetaldehyde	+	+	17
Propionaldehyde	+	+	14
Acetone	2	2	113
Methyl vinyl ketone	—	+	4
Methanol	—	+	5
Trimethyl amine	+	+	+
Dimethyl sulfide	+	—	65
Methyl ethyl sulfide	+	+	8
Dimethyl disulfide	—	+	32
Benzene	+	+	2
Toluene	+	+	2
Hexane[b]	+	+	16
Heptane[b]	—	+	2
Octane[b]	—	—	+
Chloroform[b]	+	+	3
Tetrahydrofuran[b]	+	+	+

[a] Corresponds to the amount of each compound in the gas fraction distillable at −80°C and condensable at −140°C from samples receiving 0, 0.1 or 3.0 Mrads. + Indicates detected at <1 ppb; — indicates not detected.

[b] This compound is regarded as being derived from the irradiation of the end-sealing material of the cans.

Despite the preponderance of air in the headspace gas, determinations of hydrogen and carbon dioxide were readily made. The data show that the former is produced entirely from the irradiation (1.6 ppm at 3.0 Mrads), but that as much of the latter is present prior to irradiation (31 ppm) as is produced at high doses (75 ppm found in 3.0 Mrads sample). The number of moles of both gases formed at a specific dose is approximately the same and, based on statistical analysis, increases linearly with dose.

Sixteen other compounds were extracted from the codfish, and in some cases their formation could be examined readily for dependence on dose. For example, benzene and propionaldehyde (Fig. 1) which are known to be radiolytic products from fish, show linear concentration-dose relations up to 3 Mrads. Although hexane and heptane show this dependence as well, they are derived presumably from the concomitant irradiation of the end-sealing compounds of the can. Acetone and dimethyl sulfide on the other hand are formed in irradiated fish, and their concentrations appear to level off above 1 Mrad. Whether this leveling off is due to competing secondary reactions or loss of these more plentiful compounds during fractionation cannot be ascertained at this time. All of these identified products (even if detected in only trace quantities) are listed in Table 1 for the control, the 0.1 Mrad sample, and the 3.0 Mrad sample.

Significance of the chemistry and wholesomeness data

Based on the above mechanism and the experimental data, it is reasonable to expect that the radiolytic products formed at the 3 Mrad level would correspond to the products formed in the 0.1 Mrad region, making an extrapolation from high to low dose responses possible. The increase in concentration with increasing dose for the products that were analyzed should be representative of expected responses for most nonvolatile radiolytic products as well. Most importantly, all of the radiolytic products for which a dose dependence could be evaluated show a linear increase up to at least 0.6 Mrad, implying a constancy in the relative amounts of such products, and *any* radiolytic product found at 0.1 Mrad was found in a *higher* concentration at 3.0 Mrads. Consequently, since a positive biological response is normally proportional to the concentration of the components in the test sample (Goodman and Gilman, 1965; Ariens et al., 1964), then if any deleterious effects were found for fish irradiated at, for example, 0.2 Mrad, they would be even more evident at 3 Mrads (see Fig. 2A).

Comparison of three feeding studies on irradiated codfish and haddock provides some insight into the physiological significance of increasing the concentrations of radiolytic products. The first study, conducted by Alexander and Salmon (1959) from 1957 to 1959, involved irradiating codfish in evacuated containers at ambient temperature to 2.8 and 5.6 Mrad doses, storing for 3 months, and feeding a diet of 35% and 50% irradiated fish to rats and dogs, respectively. The second more recent study on codfish, done by Ley (1974), involved freezing the fish at sea, vacuum sealing the skin-on fillets in nylon-polyethylene packages, irradiating at 0°C to a 0.6 Mrad dose, storing for 12 days at 0–4°C, steam cooking and cooling before mixing with other ingredients and feeding a 45% irradiated fish and 55% rat cake powder diet to mice and rats. The third study, conducted by Procter et al. (1971), dealt with irradiated haddock and investigated its carcinogenic potential in mice. This study involved sealing haddock fillets in polyethylene bags, keeping on crushed ice for 1–2 days, irradiating at about 10°C to a 0.2 Mrad dose, returning to ice up to 12 days, and preparing 40% haddock diet by cooking and freeze storing 2 days prior to mixing with laboratory chow. In none of the studies was any statistically significant differences found among test and control groups with respect to health associated factors such as growth, reproduction, longevity,

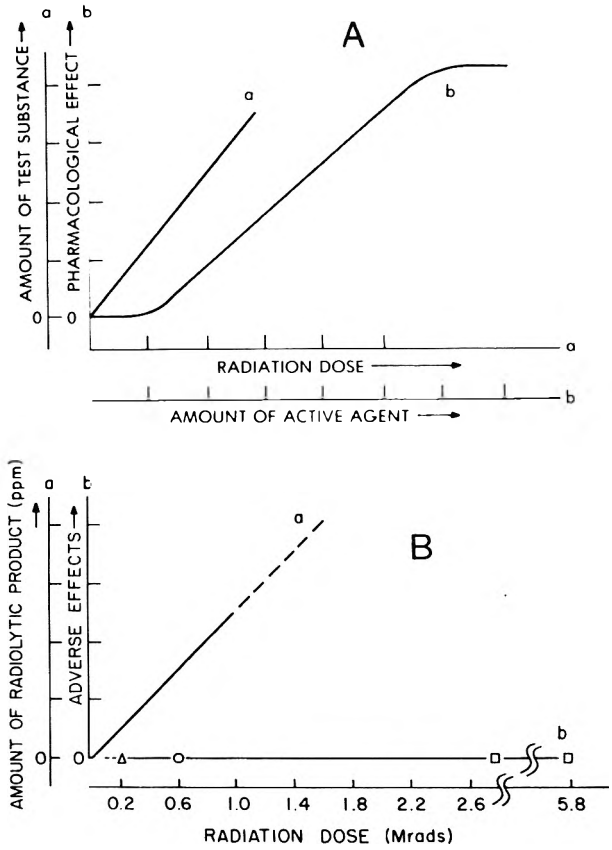

Fig. 2—A—Graphical representation of the relationships of a pharmacological response to an active agent and of concentration of radiolytic products to dose: Plot a represents the linear radiation dose dependence for formation of a hypothetical *radiolytic product having physiological activity. Curve b represents a typical pharmacological response of a test subject to an effect-inducing substance. If an active compound were formed and administered, then the responses would increase with amount after the induction level is exceeded and until the saturation level is reached.*

B—Graphical representation of the experimental relationships of radiolytic products and wholesomeness data to radiation dose in studies with fish: Plot a represents the typical dependence on dose (up to 1 Mrad) of radiolytic products found in this study for irradiated codfish. Plot b represents the wholesomeness of irradiated haddock and codfish, expressed as "adverse effects," as a function of dose. The data are from: Alexander and Salmon (□); Ley (○); and Procter et al. (△). The line is coincident with zero *effects and is extrapolated to 0.1 Mrad (dotted portion).*

hematological parameters, and tumor incidence. One concludes, therefore, that there are no adverse physiological consequences in test animals that consume radiolytic products in codfish or haddock at concentrations corresponding to irradiation doses of 0.2, 0.6, 2.8 and 5.6 Mrads, and that none should be encountered in the range 0–0.6 Mrad where product concentration is clearly proportional to dose (see Fig. 2B).

Since the data presented here indicate that the chemistry of irradiated codfish is unaffected by dose up to 0.6 Mrad (and possibly up to 3 Mrads) and since irradiation conditions associated with the animal feeding studies and the proposed ocean fish processing by the German fishing industry are similar, the conclusions from these feeding studies should apply to the clearance petition cited by Diehl (1973). Minor differences in the temperature during irradiation would not significantly influence the chemistry. [G-values for the primary species are not affected appreciably by temperature (Allen, 1961) and many of the reactions dominating the chemistry are diffusion controlled.] Differences in the availability of oxygen during processing would discernibly affect the chemistry, but not very significantly at low doses. The basis for this assessment is that products formed normally under oxic conditions would still be formed in systems where the air is only incompletely evacuated, at least until the oxygen is appreciably depleted. (The dose required to consume the residual oxygen is estimated at about 0.1 Mrad by assuming a C-value for oxygen consumption of 2 and an oxygen concentration of 2×10^{-4} M.)

CONCLUSION

THE OVERALL CONSIDERATIONS that have been made here of irradiated food chemistry, of product yields in irradiated codfish, and of the codfish and haddock wholesomeness studies lead to the conclusion that extrapolating wholesomeness results from the 2.8 Mrad, 0.6 Mrad, and 0.2 Mrad feeding studies to a 0.1 Mrad application (Fig. 2B, plot b) is trivial in this case, but very likely valid for most cases to be encountered. This conclusion should help health authorities in evaluating petitions (particularly for foods which are radiation pasteurized in the submegarad dose range) based on wholesomeness data from experiments carried out over a dose range above the doses required for commercial application. A more generalized study showing the applicability of this extrapolative approach for clearing foods which have been radiation sterilized at doses of several megarads is currently underway and will be published subsequently.

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Ms received 8/25/75; revised 11/21/75; accepted 11/24/75.

The authors are indebted to M.L. Bazinet, F. Heiligman, R.D. Jarrett, S.G. Kayser, B. MacDonald and W.G. Yeomans for their contributions to the experimental work and to A. Brynjolfsson and H.C. Johnson for their helpful comments.

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EFFECT OF FRUIT PORTION, STAGE OF RIPENESS AND GROWTH HABIT ON CHEMICAL COMPOSITION OF FRESH TOMATOES

ABSTRACT

Tomato fruits were analyzed for total and reduced ascorbic acid, pH, titratable acidity (% citric acid) and % soluble solids. Locular material from table ripe fruits were higher in total ascorbic acid and titratable acidity and lower in pH compared to pericarp tissue. In two pairs of cultivars, each pair having determinate and indeterminate growth habits in essentially the same genetic background, fruits from the indeterminate had more ascorbic acid and soluble solids than did those from determinate. When table ripe and mature green fruits (eight cultivars) were harvested on the same day, table ripe fruits were higher in reduced ascorbic acid and titratable acidity and were lower in pH. No differences in total ascorbic acid were noted between the two stages of ripeness.

INTRODUCTION

INVESTIGATIONS dealing with chemical compositional quality attributes in tomato fruits generally include measurements for % soluble solids, titratable acidity (% citric acid), pH, reduced ascorbic acid content and sugar content. Although a substantial amount of work has been directed towards investigating the influence of inherent and/or environmental factors such as cultivar (Tripp et al., 1937; Maclinn and Fellers, 1938; Currence, 1940; Crane and Zilva, 1949; Matthews et al., 1973), stage of ripeness (LoCoco, 1945, Malewski and Markakis, 1971; Yamaguchi et al., 1960), fruit size (Hallsworth and Lewis, 1944; Brown and Moser, 1941; Brown and Bohn, 1946; McCollum, 1944; Maclinn et al., 1936; Tripp et al., 1937), growing location (Hamner et al., 1942, 1945; Somers et al., 1950), field illumination (McCollum, 1944, 1946; Somers et al., 1950; Brown, 1955), date of harvest (Hamner et al., 1942; Yamaguchi et al., 1960), and postharvest holding conditions (Hamner et al., 1945; Craft and Heinze, 1954) on the reduced ascorbic acid content in tomato fruits, generalizations are difficult to make due to the presence of highly conflicting results. Due to the importance of pH for tomato processing, numerous studies have been conducted that have indicated that factors such as cultivar (Lower and Thompson, 1966; Vittum et al., 1962), harvest date (Lambeth et al., 1964; Massey et al., 1962) and stage of ripeness (Iwahori and Lyons, 1970), have a marked influence on pH values in tomato fruits. Field illumination and postharvest holding temperature, however, have negligible effects on pH (Craft and Heinze, 1954; McCollum, 1946). The soluble solids content in tomato fruit has been shown to be correlated with flavor (Simandle et al., 1966); in fact a difference of 0.2% in soluble solids can be important commercially (Lower and Thompson, 1967). Significant differences in % soluble solids have been attributed to growth habit (Emery and Munger, 1970), cultivar and harvest date (Simandle et al., 1966) but negligible effects of stage of ripeness have been noted (Massey et al., 1962). Since titratable acidity has also been shown to influence flavor, various studies have been conducted that have shown that storage conditions (Lampe and Watada, 1971), cultivars (Massey et al., 1962), fruit size (Lower and Thompson, 1967), stage of ripeness (Iwahori and Lyons, 1970) all influence this quality attribute.

The purpose of this study was to determine the influences of stage of ripeness, growth habit and fruit portion on selected chemical quality attributes from various cultivars. Special attention was given to the total ascorbic acid content since no information was available as to the influence of inherent factors such as growth habit, cultivar or stage of ripeness on this component. Furthermore, the validity of the assumption (Mapson, 1958) that 95% of the total ascorbic acid content in tomato fruit is in the reduced state was investigated.

EXPERIMENTAL

Material

Fruits from selected cultivars (Tables 1, 2 and 3) were harvested during the summers of 1973 and 1974 from East Ithaca, Freeville and Varna, New York. Table ripe fruits were analyzed in all experiments, except for the stage of ripeness experiments where mature green and table ripe fruits harvested on the same day were used.

Immediately after harvest, entire fruits or fruit tissue portions were frozen in liquid nitrogen and held at −20°C until analyzed. For each experiment, the frozen tomato fruits were crushed into a fine powder, mixed thoroughly and subsamples of the powder from bulk samples of 10–20 fruits were used to make chemical analyses for all treatment–cultivar combinations.

Compositional analyses for fruit tissue portion experiments were made on locular (placental tissue and seeds) and pericarp tissue as well as entire fruits from four cultivars (Table 3).

Data presented herein are taken from representative experiments since trends for given treatments and cultivars harvested at various times were similar for all experiments conducted.

Chemical analyses

Total ascorbic acid. Duplicate frozen subsamples of tomato fruit powder (25g) were homogenized at 1°C in a Waring Blendor in 100 ml of 1% oxalic acid for 2 min at high speed. The method of Roe and Oesterling (1944) was used for the remainder of the extraction and subsequent identification of the total ascorbic acid content. Triplicate readings for total ascorbic acid and duplicate readings for reduced ascorbic acid (below) were made for each subsample on a Bausch and Lomb spectronic 20 spectrophotometer.

Reduced ascorbic acid. Duplicate 25g frozen powder subsamples were blended at 1°C in a Waring Blendor at high speed in 100 ml of 3% metaphosphoric acid. The indophenol xylene–extraction method of Nelson and Somers (1945) was then followed for the rest of the extraction and identification of reduced ascorbic acid. The commonly used method of Loeffler and Ponting (1942) was considered undesirable because substantial variation was noted by using this method.

pH. The pH determinations were made on 25-g aliquots of homogenized subsamples with a Corning pH meter.

Titratable acidity. Titratable acidity was expressed as % citric acid equivalent and was measured by titrating 25-g aliquots with 0.1N NaOH to an end point of 8.1.

Soluble solids. A drop of homogenized sample was placed on the prism of a Bausch and Lomb desk refractometer and the % soluble solids content was read directly.

RESULTS & DISCUSSION

Composition of mature green and table ripe fruits

Ascorbic acid. Reduced ascorbic acid content in tomato fruits has characteristically been used as an indicator of the vitamin C content of these fruits (Hassan and McCollum,

Table 1—Effect of ripening on the chemical quality attributes of tomato fruits

Stage of ripeness	Cultivar							
	Fireball	Indeterminate fireball	Gardener	Determinate gardener	Heinz 1350	Cornell 72 – 35	Williamette	Cornell 72 – 98
				Total ascorbic acid (mg/100g FW)[a]				
Mature green	22.5	21.5	13.5	12.5	23.5	13.5	15.8	20.5
Table ripe	19.0	22.5 ± 0.5	14.5	12.5 ± 0.5	17.0	14.5	22.5	20.0
				Reduced ascorbic acid (mg/100g FW)[b]				
Mature green	11.9	8.8 ± 1	9.8	[c]	5.0 ± 0.2	5.0 ± 0.2	5.2	9.8
Table ripe	18.7	21.5 ± 0.5	14.5	12.6	16.0 ± 0.2	12.1 ± 0.9	19.5 ± 1.5	19.7
				pH				
Mature green	4.7	4.7	4.7	4.6	4.6	4.6	4.6	4.4
Table ripe	4.5	4.5	4.4	4.4	4.5	4.5	4.6	4.4
				Titratable acidity (% citric acid)				
Mature green	0.32	0.39	0.45	0.42	0.43	0.32	0.40	0.40
Table ripe	0.41	0.43	0.55	0.51	0.44	0.46	0.42	0.53
				% Soluble solids				
Mature green	5.5	6.5	6.5	5.5	5.5	5.6	6.0	5.0
Table ripe	5.0	6.5	6.5	5.0	5.0	5.3	6.0	6.5

[a] Mean value of three determinations; standard deviations greater than zero are shown.
[b] Mean value of four determinations; standard deviations greater than zero are shown.
[c] No determination made.

1954). It has been assumed that due to the acidic nature of the fruit, the majority of the ascorbic acid is in the reduced form and is not dehydroascorbic acid (DHA) or 2,3-diketogulonic acid (DKA) (Mapson, 1958). To establish if this assumption were correct, mature green and table ripe fruits were harvested on the same day and were subsequently analyzed for total and reduced ascorbic acid.

For all cultivars tested, the reduced ascorbic acid content in mature green fruits was lower than that in table ripe fruits; the reduced ascorbic acid level of mature green fruits ranged from 27–68% of the value for table ripe fruits for the various cultivars tested (Table 1). These findings are consistent with the works of several other workers (LoCoco, 1945; Malewski and Markakis, 1971).

Values for total ascorbic acid from mature green and table ripe fruits did not follow the above trend. In fact, seven of the eight cultivars showed little or no difference between the two stages of ripeness (Table 1). These observations suggest that the levels of DHA and/or DKA were high in the mature green fruits and that they steadily decreased during ripening either by reduction to ascorbic acid or by oxidation to other intermediates. Our results show negligible DHA and/or DKA in the ripe fruits, thus suggesting the possibility that the destruction of ascorbic acid is retarded by metabolites which inhibit ascorbic acid oxidation. This speculation is intriguing and parallels observations by Hooper and Ayres (1950) who found such substances in black currants.

Table 1 permits comparison of reduced and total ascorbic acid values for either stage of ripeness. For table ripe fruits, little or no difference between reduced and total ascorbic acid was noted, whereas substantial differences were observed for mature green fruits. In mature green fruits, the reduced ascorbic acid content ranged from 21–73% of the total ascorbic acid values for the eight cultivars tested. As a check on the procedures employed above for the total and reduced ascorbic acid analyses, mature green and table ripe tomato fruits were harvested and immediately analyzed without holding at −20°C and the same above trends were noted. Additionally, mature green and table ripe fruits were extracted in either 1% oxalic acid or 3% metaphosphoric acid and the % recovery of exogenously added ascorbic acid was found to be 90–95% for the two stages of ripeness and both extractants. These checks indicated that no differential changes took place between mature green and table ripe fruits during reduced or total ascorbic extractions and/or holding at −20°C in the frozen state. In light of the above observations, if reduced ascorbic acid is used to estimate the vitamin C content of mature green tomato fruits and other plant tissues, then consideration should be given to the possibility that inherent factors could alter the ascorbic acid content of the fresh product and hence the apparent level of the vitamin.

Titratable acidity and pH. For all cultivars studied, mature green fruits were lower in titratable acidity than table ripe fruits with the greatest differences between stages of ripeness noted in the following cultivars: Fireball, Gardener, Determinate Gardener, Heinz 1350, Cornell 72-35 and Cornell 72-98 (Table 1). The reason that these results do not parallel the observations of others (Iwahori and Lyons, 1970; Hall, 1966) could be due to the fact that we harvested the fruits on the same day, used different cultivars and imposed no postharvest holding conditions.

For six of eight cultivars tested, the pH values were slightly more acid in table ripe than mature green fruits. These results were consistent with those of Kaski et al. (1944) but in contrast with those of others (Iwahori and Lyons, 1970). The possibility that reduced ascorbic acid is oxidized preferentially in mature green relative to table ripe fruits as a result of differences in pH is thus not very likely.

Soluble solids. As reported by others using different cultivars and ripening conditions, (Hall, 1966), no appreciable differences in soluble solids were noted in mature green or table ripe fruits from seven of eight cultivars tested.

Fruit composition as influenced by growth habit

Comparison of chemical quality attributes in fruits taken from plants with either determinate or indeterminate growth habits were made by using two pairs of cultivars with essentially the same genetic background. Indeterminate Fireball had been backcrossed eight times to the standard determinate Fireball while determinate Gardener had been backcrossed eight times to Gardener which is indeterminate. This procedure gives on the average well over 99% similarity in genotype except for the growth habit genes. On determinate plants, clusters of fruit are separated by one or, occasionally, two leaves while on indeterminate plants clusters are mostly three leaves apart.

Ascorbic acid. Several investigators (Somers et al., 1950) have shown that shaded fruits are lower in reduced ascorbic acid than unshaded ones. Since more shading of fruits from leaves did occur on fruits grown on indeterminate vines, we had hypothesized that lower reduced ascorbic acid levels would be noted in these fruits relative to ones grown on determinate vines. The opposite trend, however, was observed. Fruits from indeterminate Fireball plants contained more total and reduced ascorbic acid than fruits from determinate vines. For the cultivar Gardener, fruits analyzed from indeterminate plants were either equal to or greater than fruits from determinate plants (Table 2).

% Soluble solids. As noted previously (Emery and Munger, 1970), indeterminate plants were consistently higher in % soluble solids than were determinate ones for both cultivars (Table 2). Greater photosynthetic capabilities in the indeterminate plants, due to their increased leaf number, could feasibly allow for greater photosynthate movement to the fruit and a higher % soluble solids level in these fruits. McCollum and Skok (1960) demonstrated that photosynthates were translocated from leaves to fruits and that exogenously applied glucose to the leaf was translocated to fruits. Therefore, increased amounts of ascorbic acid precursors such as glucose in fruits from indeterminate vines could be linked with enhanced ascorbic acid synthesis and this is currently under investigation.

Fruit portion

Titratable acidity and pH. No consistent trends or differences in pH or titratable acidity could be attributed to growth habit (Table 2).

Total ascorbic acid. Relative to pericarp tissue, the locular tissue contained significantly more total ascorbic acid for all cultivars studied (Table 3). The total ascorbic acid levels in locular material were 37, 37, 20 and 37% higher than pericarp tissue for cultivars Supersonic, Glamour, Springset and Jet Star respectively (Table 3). These data suggest that plants that are bred to bear fruits with a higher proportion of locular material to pericarp tissue should have a correspondingly higher total ascorbic acid content on a per fruit basis. These data are in agreement with the work of Maclinn and Fellers (1938) who showed that reduced ascorbic acid was concentrated in the gelatinous material about the seed but in contrast with the results of McCollum (1944) who reported that, depending on the amount of sunlight, wall tissue was equal to or greater than placental tissue in reduced ascorbic acid.

Titratable acidity and pH. As reported by McCollum (1956), the titratable acidity was substantially higher in locular tissue relative to pericarp tissue (Table 3). For cultivars Supersonic, Glamour, Springset and Jet Star respectively, the titratable acidity was 50%, 43%, 48% and 48% higher in the locular material compared to that of the pericarp tissue. For three of the four cultivars studied, the pH values were more acidic in the locular material.

Soluble solids. Cultivar differences were noted in the soluble solids content of the two portions (Table 3). For example, pericarp tissue of Springset had a soluble solids level of 4.75 whereas Glamour had a 6.50 soluble solids level. Furthermore, differences in soluble solids between tissue portions were dependent on the cultivar. For instance, the % soluble solids content in pericarp tissue was equal to the locular material for the cultivars Supersonic and Glamour, but much less than the locular material for Springset and Jet Star.

Table 2–Influence of growth habit on the chemical quality attributes of tomato fruits

Cultivar	Growth habit	
	Determinate	Indeterminate
	Total ascorbic acid (mg/100g FW)[a]	
Fireball	19.0	22.5 ± 0.5
Gardener	12.5 ± 0.5	14.5
	Reduced ascorbic acid (mg/100g FW)[b]	
Fireball	18.7	21.5 ± 0.5
Gardener	12.6	14.5
	pH	
Fireball	4.5	4.5
Gardener	4.4	4.4
	Titratable acidity (% citric acid)	
Fireball	0.42	0.42
Gardener	0.51	0.55
	% Soluble solids	
Fireball	5.8	6.5
Gardener	5.0	6.5

[a] Mean value of three determinations; standard deviations greater than zero are shown.
[b] Mean value of four determinations; standard deviations greater than zero are shown.

Table 3–Chemical quality attributes of tomato fruit tissue portions

Cultivar	Pericarp	Locular material	Entire fruit
	Total ascorbic acid (mg/100g FW)[a]		
Supersonic	13.5	21.3	15.2 ± 0.5
Glamour	11.3	18.0	12.5
Springset	15.2	19.0	15.2± 0.5
Jet Star	11.3	18.0	11.3
	pH		
Supersonic	4.4	4.3	4.3
Glamour	4.5	4.4	4.5
Springset	4.4	4.3	4.4
Jet Star	4.5	4.5	4.4
	Titratable acidity (% citric acid)		
Supersonic	0.41	0.82	0.54
Glamour	0.40	0.70	0.46
Springset	0.43	0.82	0.46
Jet Star	0.38	0.73	0.47
	% Soluble solids		
Supersonic	6.25	6.25	6.00
Glamour	6.50	6.50	7.00
Springset	4.75	6.25	5.25
Jet Star	6.00	7.00	6.00

[a] Mean value of three determinations; standard deviations greater than zero are shown.

Twelve cultivars were studied and differences in composition between cultivars were noted (Tables 1 and 3). It should be kept in mind, however, that in these investigations the influence of harvest date on composition could not be separated out due to the fact that some cultivars were early while others were middle and late season producers of tomato fruits. Thus, whether differences in chemical quality attributes among cultivars were exclusively a function of genetic or environmental factors could not be sorted out in this study.

Ascorbic acid. Total ascorbic acid levels ranged from a low of 12 mg/100g FW for Glamous to highs in the neighborhood of 20 mg/100g FW for Fireball, Heinz 1350, New Yorker, Williamette and Cornell 72-98. Reduced ascorbic acid levels in the cultivars tested followed the same trend; however, as noted previously, this generalization could be made only for table ripe fruits (Table 1).

pH and Titratable acidity. The pH values for the various cultivars studied ranged from 4.35 for Supersonic to 4.7 for Jet Star. Interestingly, six of the twelve cultivars tested had pH values of 4.5 or greater which could possibly be a concern to the processing industry. Marked differences in titratable acidity were also noted. The range for the cultivars analyzed was from 0.31 for Jet Star to 0.56 for Gardener (Tables 1 and 3).

Soluble solids. Soluble solids varied greatly with cultivar, with Glamour, indeterminate Fireball and Gardener having values of 7.0, 6.5 and 6.5, respectively, and Springset and determinate Gardener having values of 4.75 and 5.0, respectively.

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Ms received 9/16/75; revised 11/17/75; accepted 11/24/75.

Presented at the 35th Annual Meeting of the Institute of Food Technologists, Chicago, Ill., June 8—12, 1975.

Contribution of the Dept. of Vegetable Crops, Cornell University, Ithaca, New York. Paper No. 703.

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SYNERGISTIC TOXICITY OF FOOD ADDITIVES IN RATS FED A DIET LOW IN DIETARY FIBER

ABSTRACT

Immature male rats were fed a purified, low-fiber diet to which were added supplements of 2% sodium cyclamate, 2% FD&C Red No. 2 and 4% polyoxyethylene (20) sorbitan monostearate individually or in combination. Whereas supplements of any one of the three food additives indicated above had little if any deleterious effect, combined supplements of sodium cyclamate and FD&C Red No. 2 resulted in a marked retardation in weight increment, an unthrifty appearance of the fur, alopecia and extensive diarrhea with watery and mushy stools. Concurrent administration of all three food additives indicated above resulted in a further retardation in weight increment and death of all test animals within an experimental period of 14 days. The deleterious effects obtained on the latter diet could be largely counteracted by the concurrent administration of various dietary fiber-containing materials. Blond psyllium seed powder was particularly potent in this regard. The protective factor or factors therein was distinct from any of the known nutrients and from cellulose per se.

INTRODUCTION

AN EXTENSIVE LITERATURE is available indicating that a number of food additives are toxic when fed individually in conjunction with a purified, low-fiber diet at dosages which have little if any deleterious effect when fed with a natural food stock ration or with purified diets containing various dietary fibers (Chow et al., 1953; Ershoff, 1972; Ershoff and Thurston, 1974; Ershoff, 1974; Ershoff and Marshall, 1975). In the present communication data are presented indicating that some food additives have a synergistic toxic effect when incorporated in a purified, low-fiber diet at dosages which have little if any adverse effect when fed at the same dosage level individually.

EXPERIMENTAL

THE BASAL PURIFIED, low-fiber diet employed in the present study consisted of sucrose, 66%; casein (Vitamin Free Test Casein, Teklad Test Diets, Madison, Wisc.), 24%; salt mixture (Mineral Mix, U.S.P. XVII, Teklad Test Diets, Madison, Wisc.), 5%; cottonseed oil, 5%; and the following vitamins per kg of diet: thiamine hydrochloride, 5 mg; riboflavin, 5 mg; pyridoxine hydrochloride, 5 mg; calcium pantothenate, 50 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; para-aminobenzoic acid, 200 mg; inositol, 400 mg; biotin, 1 mg; folic acid, 5 mg; vitamin B_{12}, 150 μg; 2-methyl, 1-4 naphthoquinone, 5 mg; choline chloride, 2g; vitamin A, 5,000 U.S.P. units; vitamin D_2, 500 U.S.P. units; and alpha-tocopheryl acetate, 100 mg. The vitamins were added in place of an equal amount of sucrose. In the initial experiment 96 male rats of the Sprague-Dawley strain were selected at 21–23 days of age and an average body weight of 50.4g (range 46–54g) and were divided into eight comparable groups of 12 animals each. One group was fed the basal purified, low-fiber diet indicated above. The remaining groups were fed the purified, low-fiber diet to which were added supplements of 2% of the nonnutritive sweetener, sodium cyclamate, 2% of the food dye, FD&C Red No. 2 (amaranth), and 4% of the nonionic surface-active agent, polyoxyethylene (20) sorbitan monostearate (Tween 60). These were added individually or in combination. The test diets are indicated in Table 1. The sodium cyclamate, FD&C Red No. 2 and polyoxyethylene (20) sorbitan monostearate were incorporated in the diets in place of an equal amount of sucrose. Animals were placed in galvanized cages with raised screen bottoms (three rats per cage) and were provided the test diets and water ad libitum. Animals were fed daily and all food not consumed 24 hr after feeding was discarded. Feeding was continued for 14 days or until death, whichever occurred sooner.

Tests were next conducted on the comparative efficacy of various supplements in counteracting the toxic effects induced in rats by feeding a purified, low-fiber diet containing 2% sodium cyclamate + 2%

Table 1—Effects of sodium cyclamate, FD&C Red No. 2 and polyoxyethylene (20) sorbitan monostearate fed individually and in combination on the weight increment of immature male rats fed a highly purified, low-fiber

Diet (12 animals per group)	Avg gain (g) in body wt after following days of feeding:	
Dietary group[a]	7 days[b]	14 days[b]
Basal purified diet	37.7 ± 2.2[c]	86.8 ± 3.7[c]
Basal purified diet + following supplements:		
2% sodium cyclamate[d]	34.7 ± 2.6	78.9 ± 4.3
2% FD&C Red No. 2[e]	31.9 ± 2.4	75.7 ± 4.9
4% polyoxyethylene (20) sorbitan monostearate[f]	30.4 ± 2.5	65.3 ± 5.8
2% sodium cyclamate + 2% FD&C Red No. 2	9.2 ± 1.7	36.2 ± 3.2 (9)
2% sodium cyclamate + 4% polyoxyethylene (20) sorbitan monostearate	17.1 ± 1.3	54.4 ± 3.2
2% FD&C Red No. 2 + 4% polyoxyethylene (20) Sorbitan monostearate	22.0 ± 1.2	63.1 ± 2.5
2% sodium cyclamate + 2% FD&C Red No. 2 + 4% polyoxyethylene (20) sorbitan monostearate	5.8 ± 1.5 (8)	

[a] The average initial body weight of rats in the various groups was 50.4 g.
[b] The values in parentheses indicate the number of animals which survived and on which data are based when this number was less than the original number per group.
[c] Standard error of the mean
[d] The sodium cyclamate employed in the present experiment was kindly provided by Dr. Ronald G. Wiegand of Abbott Labs, North Chicago, Ill.
[e] FD&C Red No. 2 Certified (Amaranth), 94% pure dye, H. Kohnstamm & Co., New York, N.Y.
[f] Atlas Tween 60, McKesson Chemical Co., New York, N.Y.

FD&C Red. No. 2 + 4% polyoxyethylene (20) sorbitan monostearate. Sixty-six male rats of the Sprague-Dawley strain were selected at 21–23 days of age and an average body weight of 48.8g (range 43–57g) and were divided into 11 comparable groups of six animals each. These were fed the diets indicated in Table 2. The food additives and test supplements were incorporated in the basal purified diet in place of an equal amount of sucrose. The experimental procedure was similar to that employed above.

RESULTS & DISCUSSION

FINDINGS indicate that food additives which when fed individually with a purified, low-fiber diet had little if any deleterious effect at the dosage administered had a marked toxic effect when fed at the same dosage levels concurrently. Differences were observed, however, in the effects of different additives in this regard. Thus, whereas sodium cyclamate and FD&C Red No. 2 when fed at a 2% level and polyoxyethylene (20) sorbitan monostearate when fed at a 4% level as the sole supplement in a purified, low-fiber diet had little if any deleterious effect in the immature rat, combined supplements of 2% sodium cyclamate + 2% FD&C Red No. 2 resulted in a marked retardation in weight increment (particularly during the first week of feeding), an unthrifty appearance of the fur (which was matted and infiltrated with FD&C Red No. 2), varying degrees of alopecia and extensive diarrhea with watery and mushy stools. Three of the 12 rats in this group succumbed during the 2nd week of feeding. Gain in body weight was also retarded and lack of grooming and diarrhea also occurred although to a lesser extent in rats fed the purified, low-fiber diet supplemented with 2% sodium cyclamate + 4% polyoxyethylene (20) sorbitan monostearate. Rats fed the purified, low-fiber diet supplemented with 2% FD&C Red No. 2 + 4% polyoxyethylene (20) sorbitan monostearate, however, did not differ significantly in weight increment or appearance from rats fed the purified, low-fiber diet + 4% polyoxyethylene (20) sorbitan monostearate alone. Combined supplements of the three food additives indicated above (i.e., 2% sodium cyclamate + 2% FD&C Red No. 2 + 4% polyoxyethylene (20) sorbitan monostearate), however, were markedly toxic with all animals in this group succumbing during the experimental period of 14 days in addition to exhibiting a severe retardation in weight increment, a lack of grooming, extensive diarrhea and watery and mushy stools. The average weight increment of rats in the various groups and the number of animals surviving in each group after 7 and 14 days of feeding are summarized in Table 1. Food consumption was not determined for rats in the various groups. It was readily apparent, however, from gross inspection of the food cups that compared to other groups, food consumption was markedly reduced in rats fed the purified, low-fiber diet plus 2% sodium cyclamate + 2% FD&C Red No. 2 and rats fed the latter diet + 4% polyoxyethylene (20) sorbitan monostearate. In view of the marked diarrhea in rats fed the latter two diets, it would appear likely that in addition to a reduced food intake an imparied efficiency of food utilization associated with the diarrhea contributed to the decreased weight increment of rats in these groups.

On the basis of body weight the dosage of sodium cyclamate employed in the present study is considerably greater than that which would normally be ingested by man. Such is not the case, however, when the dosage is calculated as percent of the diet on a dry-weight basis. Three 8-oz bottles of soft drink (when cyclamates were permitted as an additive to beverages in the United States) could contain a total of 4g cyclamate. On the basis of a food intake of 400g per day on a dry-weight basis (representing a caloric intake of approximately 2400 calories), the amount of cyclamate consumed above would amount to approximately 1% of the diet. Other sources of cyclamate (when the latter was permitted as a food additive) would further increase the proportion of cyclamate in the diet. Many persons would habitually consume six bottles of soft drink per day which together with other sources of cyclamate would result in a diet containing in excess of 2% cyclamate on a dry-weight basis. The latter dosage is comparable on the basis of percent of the diet on a dry-weight basis to that employed in the present study. In the case of FD&C Red No. 2 and polyoxyethylene (20) sorbitan monostearate, however, the dosage employed in the present study was markedly greater on the basis of percent of the diet on a dry-weight basis than that which would normally be ingested by man. Available data indicate that the diet of Western man may contain as many as 2500 chemical substances which are routinely being added to our foods (Kermode, 1972). Only a fraction of these are present in any given diet; however, it is possible that sufficient food additives may be ingested to have a cumulative or synergistic toxicity when fed in conjunction with a low-fiber

Table 2—Effects of dietary supplements on the weight increment of immature male rats fed a purified, low-fiber diet containing 2% sodium cyclamate + 2% FD&C Red No. 2 + 4% polyoxyethylene (20) sorbitan monostearate (six animals per group)

Dietary group[a]	Avg gain (g) in body wt after following days of feeding: 7 days[b]	14 days[b]
Basal purified diet	30.3 ± 3.3[c]	77.2 ± 4.9[c]
Basal purified diet + 2% sodium cyclamate + 2% FD&C Red No. 2 + 4% polyoxyethylene (20) sorbitan monostearate	4.0 ± 2.2 (3)	
Basal purified diet + 2% sodium cyclamate + 2% FD&C Red No. 2 + 4% polyoxyethylene (20) sorbitan monostearate + following supplements:		
Known nutrients[d]	4.5 ± 1.5 (2)	
2½% cellulose[e]	3.0 ± 2.1 (3)	
5% cellulose	3.7 ± 2.5 (3)	19.0 ± 4.4 (3)
10% cellulose	9.6 ± 3.6 (5)	33.4 ± 6.7 (5)
2½% blond psyllium seed powder	25.2 ± 1.4	59.7 ± 3.7
5% blond psyllium seed powder	28.7 ± 2.5	70.6 ± 3.7
10% blond psyllium seed powder	29.7 ± 1.2	66.8 ± 2.6
10% carrot root powder	20.3 ± 1.3	51.4 ± 2.9
10% cabbage powder	23.8 ± 1.2	57.4 ± 2.8

[a] The average initial body weight of rats in the various groups was 48.8 g.
[b] The values in parentheses indicate the number of animals which survived and on which data are based when this number was less than the original number per group.
[c] Standard error of the mean
[d] 5% Vitamin free test casein, 5% cottonseed oil, 2.5% mineral mix (U.S.P. XVII) plus the following vitamins per kg of diet: thiamine hydrochloride, 5 mg; riboflavin, 5 mg; pyridoxine hydrochloride, 5 mg; calcium pantothenate, 50 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; para-aminobenzoic acid, 200 mg; inositol, 400 mg; biotin, 1 mg; folic acid, 5 mg; vitamin B_{12}, 150 μg; 2-methyl, 1-4 naphthoquinone, 5 mg; choline chloride, 2g; vitamin A, 5,000 U.S.P. units; vitamin D_2, 500 U.S.P. units; and alpha-tocopheryl acetate, 100 mg.
[e] Solka-Floc, Brown & Co., Boston, Massachusetts.

diet. It is highly unlikely that the three food additives tested in the present study are the only ones that have such an effect. Dozens or even hundreds of other additives may be similarly involved. Whether combinations of food additives will have a cumulative or synergistic toxicity at the dosage levels at which they are customarily present in foods, however, remains to be determined.

Present findings indicate that the toxic effects obtained in immature rats by feeding a purified, low-fiber diet supplemented with 2% sodium cyclamate + 2% FD&C Red No. 2 + 4% polyoxyethylene (20) sorbitan monostearate could be largely counteracted by the concurrent administration of various dietary fiber-containing materials. In agreement with findings in the initial experiment, the addition of 2% sodium cyclamate + 2% FD&C Red No. 2 + 4% polyoxyethylene (20) sorbitan monostearate to the purified, low-fiber diet resulted in a marked retardation in weight increment, extensive diarrhea, an unthrifty appearance with matted fur infiltrated with FD&C Red No. 2 and death of all rats in this group within an experimental period of 14 days. These effects were largely counteracted by the concurrent administration of carrot root powder or cabbage powder at a 10% level of supplementation or blond psyllium seed powder at levels of 2½%, 5% or 10% of the diet. No deaths occurred in any of the rats fed the latter supplements, and in the case of rats fed diets containing blond psyllium seed powder these animals differed but slightly from those fed the basal purified diet alone. Supplements of the known nutrients (see Table 2 for the level at which these were administered) when incorporated in the purified, low-fiber diet containing the three food additives indicated above were without protective effect. A supplement of 2½% cellulose was also without protective effect. At a 5% level of feeding, three of six rats and at a 10% level five of six rats fed the cellulose supplement survived the experimental period of 14 days although the average weight increment of rats in these groups was markedly less than that of rats fed the carrot root powder, cabbage powder or blond psyllium seed powder supplements. Inasmuch as the amount of crude fiber present in supplements of 10% carrot root powder, cabbage powder or blond psyllium seed powder was less than that provided by a supplement of 2½% cellulose (which was without activity), it is apparent that the protective factor or factors therein was not cellulose per se. Further studies are indicated, directed toward the concentration, isolation and identification of the active factor or factors in the dietary fiber-containing materials indicated above and the modus operandi of their protective effect.

The observation that some food additives have a cumulative or synergistic toxic effect when incorporated in a purified, low-fiber diet at dosages which have little if any adverse effect when fed at the same dosage level individually may be of considerable public health significance. An extensive literature is available indicating that an inadequate intake of dietary fibers constitutes one of the major deficiencies in the diet of Western man. The widespread introduction of roller milling between 1877 and 1880 followed by the growth of the food processing industry and changes in technology have been implicated as contributing to a marked reduction in the amount of dietary fiber ingested by Western man, particularly of the type present in cereals and grains (Painter and Burkitt, 1971; Robertson, 1972; Burkitt et al., 1972). Associated with this reduction in the fiber content of the diet there has been an accompanying increase in such disorders as diverticulosis, adenomatous polyps, ulcerative colitis, hemorrhoids, and cancer of the rectum and colon, conditions which are virtually nonexistent in populations subsisting on high residue diets (Cleave et al., 1969; Burkitt, 1971; Painter and Burkitt, 1971; Burkitt et al., 1972; Burkitt, 1973). In view of the low-fiber diets ingested by so many persons in the United States and other Western countries, serious questions arise as to whether doses of some food additives which may be without deleterious effect when ingested by persons on high-fiber diets may not constitute a hazard to health for a substantial portion of the population of these countries.

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Ms received 9/18/75; revised 12/9/75; accepted 12/16/75.

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EFFECT OF VARIOUS MAGNESIUM SOURCES ON BREADMAKING CHARACTERISTICS OF WHEAT FLOUR

ABSTRACT

A number of organic (lactate, acetate and citrate) and inorganic (chloride, phosphate, oxide, carbonate, sulfate and hydroxide) magnesium sources, but oxide in particular, were tested for their effect on the quality of bread made by sponge-dough, no-time dough and continuous mix procedures. When added at the level of 44.1 mg of magnesium per 100g flour, magnesium-rich sources (oxide, hydroxide and carbonate), but not the others, raised bread pH appreciably and adversely affected loaf volume and general quality including the flavor. Further studies, using sponge-dough procedure and magnesium oxide, revealed that adding magnesium to the dough instead of the sponge or using flour prefortified with magnesium, greatly improved loaf volume and overall quality; such an improvement also occurred when pH elevation, due to magnesium added to the sponge, was prevented by acetic acid added to the sponge or the dough.

INTRODUCTION

TO RESTORE some of the nutrients lost during the commercial milling of wheat and thus to reduce the risk of their deficiency in human dietary, commercial white bread, since the early 1940's, has been enriched with iron, thiamine, riboflavin and niacin (Wilder and Williams, 1944). On the basis of the information recently reviewed (Anonymous, 1974a), the potential risk of deficiency of certain additional vitamins and minerals amongst significant segments of the population seems to exist. Magnesium (Mg) which is involved in enzymatic reactions of carbohydrates, protein and energy metabolism and in the maintenance of structural and functional integrity of body tissues, is considered to be one such nutrient likely to be deficient in our diet. Fortification of cereal-grain products with Mg has thus been suggested (Anonymous, 1974a). Present work was undertaken to examine the breadmaking characteristics of a number of organic and inorganic Mg sources.

EXPERIMENTAL

VARIOUS Mg sources (reagent grade) with Mg content ranging from 9.5–58.1% (Table 2) were tested in breads (pound loaves) made with unenriched, unbromated bleached patent flour obtained from hard red winter wheat. Loaves were baked in batches of two (sponge-dough and no-time dough procedures) or six (continuous mix procedure); results thus represent the averages. Formulation and baking procedures followed are listed in Table 1. All breads were plastic-bagged after cooling on racks and evaluated the following mornings for 1–3 days. Following weight and volume (rapeseed displacement) measurements, loaves were sliced and general bread quality based on appearance (break and shred, crust and crumb color, grain, and texture) was assessed. Quality and flavor evaluation, on coded loaves, were conducted separately by three baking technologists; flavor evaluation, on a limited scale, was also made by an untrained panel of over 20 individuals.

In all studies, Mg was added at the recommended (Anonymous, 1974a) level of 44.1 mg/100g flour. Mg content of various sources was determined by atomic absorption spectrophotometry (Anonymous, 1974b) using an IL model 251 (Instrumentation Laboratory Inc.) spectrophotometer; determined values closely approximated the formula values. The pH in bread was determined by the standard AACC (1962) method. Where indicated, 45 ml of 5% v/v solution of acetic acid (reagent grade) was used to adjust pH.

RESULTS & DISCUSSION

Mg source and bread quality

All Mg sources, except hydroxide, were initially tested in bread made by the sponge-dough procedure. Results (Table 2) showed that only oxide and carbonate appeared to exert significant deleterious effects on loaf volume and quality including flavor. Inorganic Mg sources tested by two other breadmaking procedures showed similar, though less pronounced, effect due to oxide and carbonate (Table 3). This probably occurred due to the interaction of oxide and carbonate with bread ingredients with resultant increase in pH (Table 3). Mg sources other than oxide and carbonate produced breads of excellent quality, quite comparable to the control (no Mg added) bread (Tables 2 and 3). Mg content of these sources is, however, quite low (Table 2); also, the physiological availability of Mg from these sources is no higher than that from Mg-rich sources such as oxide and carbonate (Ranhotra et al., 1976). Consequently, further studies were mostly limited to examining and improving characteristics of bread fortified with Mg-rich sources especially the oxide.

Table 1—Formulation and methods of breadmaking

	Sponge-dough[a]		No-time[b]	Continuous mix[c]	
	Sponge (%)	Dough (%)	(%)	Brew (%)	Dough (%)
Flour	70	30	100	30	70
Yeast	2.5	–	5	2.5	–
Yeast food	0.5	–	0.5	0.5[d]	–
Salt	–	2	2	2.3	–
Sugar	–	6	4	2	6
Shortening	–	3	3	–	3.25[e]
Dry milk (NF)	–	2	2	2	–
Ca acid phosphate	–	–	–	0.2	–
Ca propionate	–	–	–	0.1	–
Oxidation mixture	–	–	–	–	1[f]
Water	45.5	19.5	65	66	–

[a] Sponge-dough: Sponge mix (McDuffee bowl), 1 min at 1 speed and 1 min at 2 speed (Hobart A-120 mixer); sponge time, 3.5 hr; dough mix, 1 min at 1 speed and 4 min at 2 speed; floor time, 20 min; intermediate proof, 10 min; pan proof (to template-times in Tables); bake, 20 min at 430° F.

[b] No-time: Mix (bowl and hook), 1 min at 1 speed and 11 min at 2 speed (Hobart A-120 mixer); sponge time, 30 min; intermediate proof, 10 min; pan proof (to template-times in Tables); bake, 20 min at 430° F.

[c] Continuous mix: Brew fermentation (Baker Do-maker laboratory mixer), 2.25 hr at 86° F; fermented brew and dough ingredients mixed, 2 min at 1 speed (Hobart A-200 mixer with bowl and hook); proof, 50 min; bake 18 min at 430° F.

[d] Non-phosphate type

[e] Includes flakes and emulsifiers

[f] Azodicarbonamide, 20 ppm; K-bromate, 40 ppm

Stage of Mg addition and pH adjustment

When Mg oxide and carbonate were added to the dough instead of the sponge (sponge-dough procedure), loaf volume and overall quality were greatly improved (Table 4). This occurred in spite of elevation in pH. In contrast, the addition of oxide and carbonate to the dough instead of the brew (continuous mix procedure), did not improve bread quality appreciably (Table 3). In the sponge-dough procedure of breadmaking, adding acetic acid to the sponge or the dough, fully counteracted the deleterious effect, on bread quality, of Mg oxide added to the sponge (Table 4). A similar effect, due to acetic acid, was also observed when Mg hydroxide and carbonate were added to the sponge. Although 45 ml of acid was routinely used, 15 ml appeared to be adequate (lower levels were less effective) to fully restore the loss of bread quality due to Mg oxide added to the sponge (Table 5). Although it was not tested, this is probably true for carbonate and hydroxide as well. Acid levels exceeding 45 ml (5% solution) prolonged proof time considerably, lowered pH below normal levels and greatly adversely affected overall bread quality.

Using prefortified flour

When Mg oxide-fortified flour, as in mill-fortification, was used at various sponge-dough ratios (Table 6), 70/30 ratio proved to be most effective in producing good quality bread

Table 2–Effect of various magnesium sources on bread quality (Sponge-dough procedure)

Magnesium source[a]	Specific loaf volume[b] (cu in./oz)	General bread quality (score)[c]	Flavor 1 day (score)[d]	Flavor 2 day (score)[d]	Proof time (min)
None	10.72	3.8	4.0	4.0	55
Lactate (9.5)	10.86	3.7	4.0	4.0	52
Acetate (11.4)	10.55	3.9	3.5	4.0	57
Citrate (14.7)	10.54	3.6	4.0	4.0	54
Chloride (12.4)	10.72	3.8	4.0	4.0	53
Phosphate (14.0)	10.59	3.8	4.0	4.0	52
Oxide (58.1)	9.63	2.9	3.0	3.5	57
Carbonate (24.9)	9.99	3.2	2.0	2.0	55
Sulfate (15.2)	10.72	3.8	4.0	4.0	56
Hydroxide (42.5)	–	–	–	–	–

[a] Values within parentheses indicate content (%) of magnesium; source added to the sponge.
[b] Loaf weight ranged from 16.50–16.63 oz
[c] Very good, 4; good, 3; fair, 2; and poor, 1
[d] Acceptable, 4; moderately acceptable, 3; slightly acceptable, 2; and unacceptable, 1

Table 3–Effect of various magnesium sources on bread quality (No-time and Continuous mix procedures)

Mg source[a]	Specific loaf vol[b] (cu in./oz)	General bread quality (score)[c]	Flavor 1 day (score)[d]	Flavor 2 day (score)[d]	Proof time (min)	Bread pH
No-time						
None	9.80	3.6	4.0	4.0	48	–
Chloride	9.61	3.4	4.0	4.0	47	–
Phosphate	9.70	3.7	3.0	4.0	47	–
Sulfate	10.03	3.2	4.0	4.0	48	–
Oxide	9.15	2.9	3.5	4.0	52	–
Carbonate	8.97	3.1	3.5	3.0	49	–
Continuous mix						
None	8.51	3.9	4.0	4.0	–	5.38
Chloride (B)	8.56	3.9	4.0	4.0	–	5.32
(D)	8.33	3.8	4.0	4.0	–	5.38
Phosphate (B)	8.62	3.8	4.0	4.0	–	5.43
(D)	8.37	3.2	4.0	4.0	–	5.50
Sulfate (B)	8.70	4.0	4.0	4.0	–	5.32
(D)	8.52	3.8	4.0	4.0	–	5.42
Oxide (B)	7.80	3.0	4.0	4.0	–	6.30
(D)	7.81	3.1	3.0	4.0	–	6.28
Carbonate (B)	7.70	2.4	2.0	3.0	–	6.35
(D)	8.07	2.4	3.0	2.0	–	6.42

[a] Added to the ingredients (No-time procedure) or to the brew (B) or dough (D).
[b] Loaf weight ranged from 15.25–16.75 oz
[c,d] As in Table 2

Table 4–Effect of magnesium and acetic acid on bread quality (Sponge-dough procedure)

Mg source[a]		Specific loaf vol[b] (cu in./oz)	General bread quality (score)[c]	Flavor 1 day (score)[d]	Flavor 2 day (score)[d]	Proof time (min)	Bread pH
First bake							
None		10.57	3.9	4.0	3.8	52	–
Oxide	(S)	9.79	3.2	4.0	3.5	57	6.02
	(D)	10.29	3.8	4.0	4.0	55	5.92
	(S)(A)	10.49	3.9	4.0	4.0	55	5.20
	(S)(AA)	10.32	3.8	3.5	4.0	70	5.22
Carbonate	(S)	9.78	3.6	2.0	2.0	53	–
	(D)	10.63	3.9	3.5	2.0	60	–
Second bake							
Oxide	(S)	9.73	3.6	4.0	2.0	57	6.02
	(S)(A)	10.18	3.7	4.0	4.0	56	5.22
Carbonate	(S)	9.68	3.4	4.0	4.0	53	6.02
	(S)(A)	10.14	3.6	4.0	4.0	52	5.20
Hydroxide	(S)	9.87	3.4	4.0	3.0	58	5.93
	(S)(A)	10.18	3.7	4.0	4.0	57	5.18

[a] Added to sponge (S) or dough (D) and where indicated, acetic acid also added at the expense of sponge (A) or dough (AA) water
[b] Weight ranged from 16.38–17.38 oz
[c,d] As in Table 2

Table 5–Effect of pH on quality of bread baked with magnesium oxide (Sponge-dough procedure)

Acetic acid[a] (ml)	Specific loaf vol[b] (cu in./oz)	General bread quality (score)[c]	Flavor 1 day (score)[d]	Flavor 3 day (score)[d]	Proof time (min)	Bread pH
0(+)	9.72	3.0	3.5	3.5	61	6.11
15(+)	10.91	3.7	4.0	3.5	58	5.88
30(+)	10.86	3.8	4.0	4.0	55	5.57
45(+)	10.07	3.8	4.0	4.0	54	5.23
60(+)	9.72	3.7	4.0	4.0	65	5.05
75(+)	7.67	2.6	3.0	3.0	75+	4.92
0(0)	10.45	3.6	4.0	3.5	52	5.20

[a] Added in amount indicated at the expense of sponge water along with magnesium (+)
[b] Loaf weight ranged from 16.38–17.00 oz
[c,d] As in Table 2

(sponge-dough procedure); other ratios produced quite acceptable bread also. It appears that distributing Mg between sponge and dough, instead of adding all to the sponge, does not adversely affect bread quality in spite of elevation in pH.

Table 6—Effect of magnesium oxide-enriched flour used in various sponge-dough ratios on bread quality

Flour[a]		Specific loaf vol[b]	General bread quality	Flavor	Proof time	Bread
Sponge (%)	Dough (%)	(cu in./oz)	(score)[c]	1 day (score)[d]	(min)	pH
No magnesium						
40	60	10.21	3.3	4.0	61	5.37
50	50	10.72	3.5	4.0	60	5.32
60	40	10.88	3.6	4.0	55	5.30
70	30	10.60	3.8	4.0	54	5.25
Magnesium enriched						
40	60	10.72	3.3	4.0	68	6.19
50	50	11.08	3.4	4.0	67	6.18
60	40	11.23	3.6	4.0	70	6.12
70	30	11.28	3.6	4.0	69	6.08

[a] Premixed, where indicated, with magnesium oxide
[b] Loaf weight ranged from 16.25–16.50 oz
[c,d] As in Table 2

Table 7—Flavor preference of magnesium-fortified bread[a] (Untrained panel)

Flour	Day 1	Day 3	Bread pH
Unfortified	8 (3)[b]	14 (4)	5.20
Mill-fortified	14	4	5.12
Unfortified	3 (8)	10 (8)	5.22
Bakery-fortified	14	8	5.12

[a] Bread baked by sponge-dough procedure based on 70/30 flour ratio. Where indicated, magnesium oxide was added to the flour (mill-fortified) or to the sponge (Bakery fortified). Acetic acid, at the expense of dough water, was also added to fortified breads.
[b] All numbers within parentheses indicate no preference.

Flavor evaluation

Off-flavor, due to Mg oxide and carbonate, was invariably detected irrespective of the method of baking used; it however, appeared to be slightly more intense in carbonate-enriched bread. Off-flavor improved somewhat with time and on toasting, and when pH was also adjusted, with acetic acid, to normal levels. Adjustment of pH, however, may be more important for other considerations especially under commercial production conditions. Flavor evaluation made by an untrained panel revealed that breads made with prefortified flour (mill-fortification) or with flour fortified at the sponge side (bakery fortification), were more preferred on the first than on the third day after baking in comparison to the control breads (Table 7). A large number of individuals, however, showed no preference, on either day, for sponge-fortified bread.

Mg type

In evaluating various Mg types, only slight differences were observed. For example, heavy Mg oxide yielded bread of slightly better quality while for carbonate, the light basic type was a little better. Two hydroxide types tested differed little while heptahydrate sulfate, in comparison to anhydrous sulfate, performed slightly better. Such differences, however, appear to be of little significance and may not always be detectable.

CONCLUSION

BASED on the limited information presented here, it may be inferred that Mg oxide, and probably Mg hydroxide and carbonate, can be used in suggested fortification of flour without noticeable loss of bread quality if necessary modifications in the baking procedures are made. However, the interaction of bread ingredients with added Mg and the resultant flavor profile must be examined in greater details to fully assess the potential of Mg-rich sources in a fortification program.

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Ms received 11/8/75; revised 1/15/76; accepted 1/20/76.

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MIGRATION OF MINOR CONSTITUENTS FROM FOOD PACKAGING MATERIALS

ABSTRACT

Techniques for the evaluation of potential migration of minor food packaging materials constituents are discussed with emphasis on vinyl chloride monomer (VCM) migration including recently developed methods of analysis. These methods combined with sorption isotherm theory enable calculation of the maximum allowable concentration of a potential migrant (e.g., VCM) in a packaging material corresponding to an "effective zero" migration to the contacting phase.

INTRODUCTION

THE POSSIBLE MIGRATION of minor constituents from polymeric packaging materials has become increasingly important in the last 15 yr. The migration from flexible packaging materials in contact with food would not normally include major components of the package itself but may contain minor constituents. Some of these migrants can and do affect quality of the contained product as exhibited by sensorily determinable changes (odor and/or taste) or by toxicological symptoms from ingestion. The first case is of economic importance, while the second relates directly to health hazards with carcinogens as the "worst case." Techniques for the evaluation of potential migration are discussed with emphasis on vinyl chloride monomer (VCM) migration including recently developed methods of analysis. These methods combined with sorption isotherm theory, enable calculation of the maximum allowable concentration of a potential migrant (e.g., VCM) in a packaging material corresponding to an "effective zero" migration to a contacting phase.

Thus, the dilemma of enhanced analytical sensitivity can be resolved by the corresponding enablement of measurements of appropriate sorption isotherms at concentrations sufficiently low to be ineffectual on a biological basis.

MIGRATION CONSIDERATIONS

MINOR CONSTITUENTS are present in packaging materials either intentionally or by accident. The deliberate additives are often made to obtain a desired effect on the properties of the material while the incidental residues are related to processing materials and/or operations.

The basic mechanism of migration applies to both situations, in that migration is a function of the initial concentration of the migrant in the material and to its distribution at equilibrium, between the package and its contents.

The major difference in these two cases is that in the first, the initial concentration is known and relatively constant, while in the second, it is variable according to the materials and conditions used in processing or converting raw materials into the finished package.

Toxicological considerations

The identity of the migrant can be important in situations determined by its effect on the use of the package contents. For migrants whose sensory effects occur at substantially higher levels than any toxicity effects, the "no effect level" can be quantified on the basis of sensory threshold. Where toxicity is concerned, "no effect levels" are equivalent to "no effect dosage." The toxicity question is of particular importance where carcinogenesis by a potential migrant can be demonstrated at any dosage level for an animal species, because of safety and legislative considerations. The legislative criteria are based on the Delaney Clause of the 1958 amendment to the Food and Cosmetic Act of 1938. This states that a substance is deemed unsafe... "if it is found to induce cancer when ingested by man or animal, or if it is found, *after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal*"... (Federal Food, Drug & Cosmetic Act, 1958).

The key question then is that of the appropriateness of the test used for evaluation of safety. It is not the Delaney Clause which is scientifically unsound, but rather the validity of the use of extrapolated data from extremely high dosage levels in animal experimentation, without appropriate physical/chemical data on migration, to reach decisions on the potential for carcinogenesis or other toxicity of such migrants. The regulations further state that where there is "no migration," the question of carcinogenesis by ingestion does not apply.

Thus the issue can be stated as the definition of "no migration" on a scientifically sound basis. Gilbert (1975) and Gilbert et al. (1974) postulated an "Effective Zero" migrant concentration on the basis of two assumptions. The first is that carcinogenesis is a chemical reaction obeying the laws of chemical kinetics, thus having a concentration dependent reaction rate. Therefore, at some finite concentration of the critical reactant, the probability of an occurrence of a reaction would be essentially zero within a biologically defined time period, such as a human life. Similar postulates have been advanced by Hutchinson (1964) and Dinham (1972). A recent set of calculations of "no risk dosage" have been made by Schneiderman et al. (1975) based on the data of Maltoni (1974) on carcinogenesis in mice from the inhalation of vinyl chloride and using various models for dosage/response relations.

Hefner et al. (1975) have indicated that in rats the metabolic fate of inhaled VCM was concentration dependent. More recently, Watanabe et al. (1975) have shown that a similar if not identical relation to concentration exists for ingested VCM in rats with the hypothesis that the detoxification of VCM is a saturable process related to the normal sulfhydryl related respiratory system.

Migrant sorption/desorption

The second assumption of the "Effective Zero" migration postulate is that migration is essentially zero at some finite but low migrant concentration, when transfer occurs from a matrix exhibiting sorption at active sites. This case would result in concentration dependent partition between polymer and extracting medium, whereby the mass transfer would be greatly hindered for finite time at such low concentrations and with only moderately different masses in the transfer phases.

The familiar set of parabolic curves characteristic of Fickian diffusion are generally considered as representative of migration in packaging application. They can be found when no "active sites" are present for interaction of solute with solvent (e.g., polymer), or where at higher initial concentrations, the major fraction of the solute is in an "unbound" or "clustered" state—the conditions for multimolecular layering.

Thus the second assumption applies best for those migrants where residues or initial additives are at relatively low levels, e.g., in concentrations below 10 ppm, or where very high partition ratios restrict migration, as in the case of poor solvency in the extracting medium.

In the past, difficulties of analysis for migration at very low concentration of migrants have led to conclusions based only on data extrapolated from high initial concentrations to very low ones. A recent paper on vinyl chloride transfer (Daniels and Proctor, 1975) is an example.

We have for the past 10 years been concerned with establishing sensitive, accurate methods for measuring migration at low levels characteristic of sensory or toxicity thresholds and in using such data to

establish the quantitative relations of the desorption phenomena concerned.

The method used for quantifying volatilizable residues (Wilks and Gilbert, 1968) is based on thermal distillation in an enclosed volume followed by quantification by gas chromatography. The migration of such residues to can contents and to food in pouches has been followed by Wilks and Gilbert (1972).

Another study in our laboratory was concerned with an intentional additive, organotin (OT), present at relatively high levels of from 1–3% of the base polyvinyl chloride polymer (Gilbert et al., 1975). At these high levels, certain other considerations can also modify the mechanism of desorption. It has long been known, following Gibbs, that for solutes which lower the surface energy of the solvent phase (here the polymer),

$$u = \frac{-c}{RT} \cdot \frac{d\gamma}{dc}$$

where u is the excess of solute in the surface layer per unit area of surface, c is concentration of the solute in the bulk phase, γ is surface tension, T is temperature, and R is the gas law constant.

Thus for any substance which decreases γ in proportion to c, u is positive so that the surface concentration is greater than in the bulk.

Many design additives such as slip, antiblock and wetting agents are effective in lowering surface tension and "plate out" on the surface. It can readily be seen that for food and simulating solvents which provide limited solubility for such additives, or for a restricted migrant phase, the determining factor in migration is often limited to that amount present on the surface, particularly for the actual use time of packaged food.

This effect was clearly shown in data by Downes and Gilbert (1975) on migration of OT from washed and unwashed PVC surfaces. The data showed delayed and lowered migration from washed surfaces, particularly where face to face contact was maintained after washing eliminates a free surface prior to contact with new solvent. The attainment of equilibrium in poor solvents, such as water, therefore, cannot be considered as one controlled solely by internal or bulk diffusion of OT in the polymer as predicted for Fickian equilibrium.

The removal of residual solvents from polymers is an illustration of a nonintentional minor constituent. We have been aware for some time that sensory thresholds for such solvents are not reached in packaged products at very low residue levels, despite their demonstrated presence by very sensitive analytical techniques (Wilks and Gilbert, 1972). Moreover, these residues persist in packaging materials for a relatively long time after manufacture. The converted products containing such persistent residues have been subjected to very short periods (below 1 min) of moderately high temperature (50–100°C) in drying ovens and then stored and used at ambient temperatures (except for heat processed food in pouches and cans).

According to Wilks and Gilbert (1972), the Fickian diffusion based concepts are not valid at levels of trace residues when polymer/solute bonding can occur. The degree of extraction is more related to the ability of the solvent to desorb chemically the residue from its active site in the polymer matrix by migration of solvent to the solid phase as distinct from the case where air is the sink for migration. Thus nonlinear desorption is predicted at low migrant concentration.

Considerations such as these provide supportive evidence for the second assumption in the above concept of an "Effective Zero" concentration of potential migrants in packaging materials. The recent findings for carcinogenesis by vinyl chloride in an animal species by experiment, and the circumstantial evidence for a similar action in humans (Selikoff and Hammond, 1975) have made of extreme economic importance the focused question of the validity of the experimental tests for migration of potential carcinogens from package to food contents.

THEORETICAL TREATMENT OF PARTITION DISTRIBUTION

THE SECOND ASSUMPTION of the "Zero Effective" transfer postulate has been verified experimentally by Morano et al. (1975) who studied the distribution of VCM between a polymer (PVC) and a fluid contacting phase. Partition values were obtained by scrption.

Determination of partition coefficient

Commercial PVC resin in the powder form was stripped of residual monomer by aeration to below 10 ppb, as determined by GC/headspace analysis. Serum bottles of 30 ml capacity were used for the sorption studies. Samples of 16g of stripped resins were used for water and hexane studies while 12g of resin were employed for the cottonseed oil (Crisco) studies. Twenty ml of solvent phase were added, stoppered to avoid headspace and appropriate additions made by injection of vinyl chloride solutions in the specific liquid.

After 1 hr of mixing, the chambers were centrifuged. After separation of resin, 10 $\mu\ell$ aliquots of the liquid were injected for GC analysis.

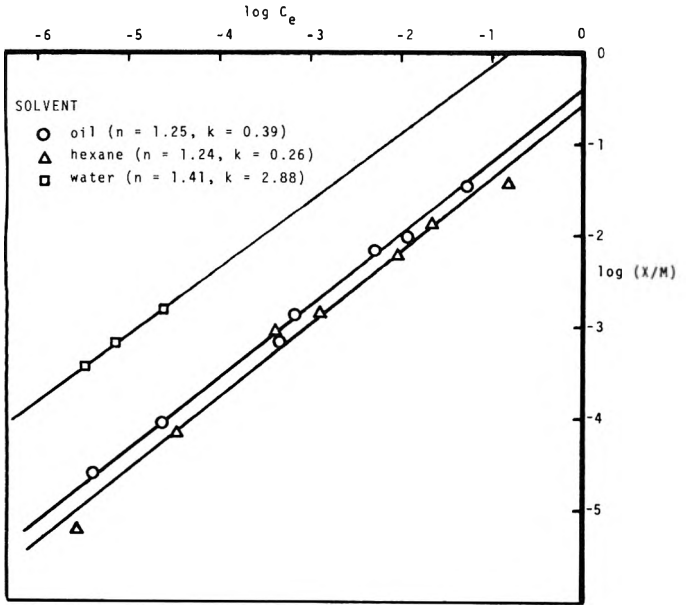

Fig. 1—Freundlich plot of the isotherm for VCM adsorbed on unplasticized PVC.

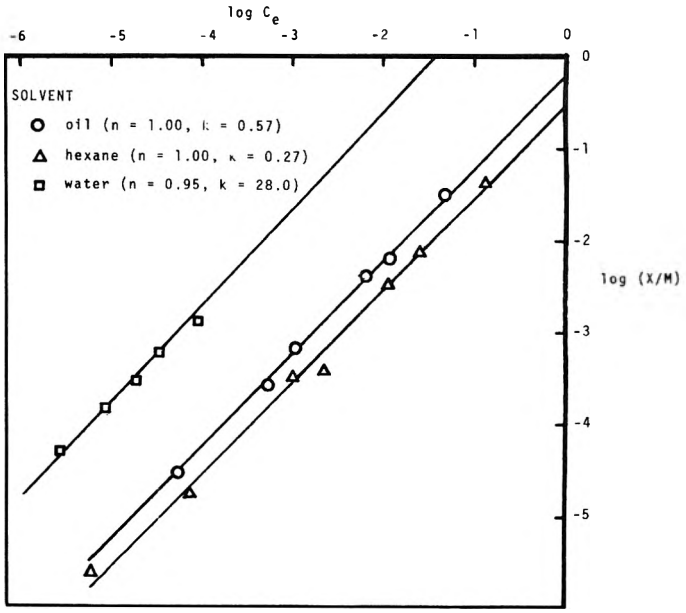

Fig. 2—Freundlich plot of the isotherms for VCM adsorbed on plasticized PVC.

The partition to resin was calculated from the decrease in concentration in the liquid phase. Appropriate control experiments were carried out to establish that no measurable losses of VCM from the sorption chambers occurred during the course of the experiments.

The partition of the monomer from polymer to the food simulating solvents (hexane and water) and to edible oil was shown to exhibit a nonlinear relation at low levels of monomer (ppm), where the polymer is unplasticized. The partition ratio is not constant and appears to become exponential toward the polymer when the initial concentration of monomer is low. While the data for partition to water shows only a linear dependence to concentration at the concentrations studied, it is anticipated that examination at lower concentrations will also show an exponential partition function for this solvent for VCM.

Partition distribution in terms of a sorption isotherm

Morano et al. (1975) has interpreted the data as a combination of mass action laws for chemisorption with normal distribution law for the "unbound" excess monomer, but no quantitative interpretation was made. The Vieth-Sladek treatment of a Dual Mode Sorption would apply here for the general case as shown by Berens (1974).

A classical treatment of these data can be made on the assumption that the desorption of a monomer from a polymer by an extracting solution is in many respects similar to that of sorption of solutes from solutions by an active solid reagent such as charcoal. The Freundlich equation can be modified to apply to this case as shown in Figures 1 and 2. Tables 1 and 2 show that hexane and oil have practically identical desorption isotherms from either plasticized or unplasticized resins. Water differs from the organic solvents in both resin types primarily in a higher K value. This value is slightly higher for the unplasticized resins.

A theoretical treatment of these data follows from either Freundlich or Langmuirian isotherms.

(1) $(X/M)\,n = KC_e$

n and K are arbitrary constants

C_e = migrant conc in soln at equilibrium = W_s/MR

(2) $W_p = M(K\,C_e)^{1/n}$

M = mass of polymer

R = soln/polymer mass ratio

$X = W_p$ = wt of migrant in polymer at equilibrium

W_s = wt of migrant in soln at equilibrium

W_o = original wt of migrant in polymer = $W_p + W_s$

The usual Freundlich plot of log X/M vs C_e provides values for n and K as the reciprocal of the slope and the y intercept respectively.

Similarly, the Langmuir isotherm (BET Type I) (Brunauer et al., 1938, 1940) can be used for the low concentration range of these data with the relation:

(3) $\dfrac{V}{V_M} = \dfrac{CX}{1 + C\,X}$

where α = "active" fraction in polymer

Let $\dfrac{V}{V_M} = \dfrac{W_p}{\alpha M}$

$S = W_{sat}$ = wt of migrant in a saturated soln in food as corresponding phase

and $X = \dfrac{W_s}{S}$

(4) $W_p = \dfrac{\alpha MC\,W_s}{S + C\,W_s}$

C = energy term related to bonding between polymer and migrant at isotherm

Table 1—Data for Freundlich plot of the isotherm for VCM adsorbed on unplasticized PVC

Solvent	C_e (g/g)	X/M (g/g)	log C_e	log (X/M)
Oil	5.55×10^{-2}	3.48×10^{-2}	−1.256	−1.458
	1.14×10^{-2}	9.67×10^{-3}	−1.943	−2.015
	5.02×10^{-3}	7.02×10^{-3}	−2.300	−2.154
	6.95×10^{-4}	1.37×10^{-3}	−3.158	−2.864
	4.54×10^{-4}	6.69×10^{-4}	−3.343	−3.174
	2.30×10^{-5}	8.42×10^{-5}	−4.637	−4.075
	4.00×10^{-6}	1.62×10^{-5}	−5.398	−4.791
Hexane	1.53×10^{-1}	3.76×10^{-2}	−0.815	−1.424
	2.20×10^{-2}	1.37×10^{-2}	−1.658	−1.864
	9.33×10^{-3}	5.94×10^{-3}	−2.030	−2.226
	1.33×10^{-3}	1.39×10^{-3}	−2.878	−2.856
	4.17×10^{-4}	9.19×10^{-4}	−3.380	−3.037
	3.33×10^{-5}	6.62×10^{-5}	−4.477	−4.179
	2.73×10^{-6}	5.89×10^{-6}	−5.564	−5.230
Water	2.43×10^{-5}	1.50×10^{-3}	−4.614	−2.824
	7.10×10^{-6}	6.75×10^{-4}	−5.149	−3.171
	7.20×10^{-6}	6.75×10^{-4}	−5.143	−3.171
	3.28×10^{-6}	3.56×10^{-4}	−5.485	−3.449

Table 2—Data for Freundlich plot of the isotherm for VCM adsorbed on plasticized PVC

Solvent	C_e (g/g)	X/M (g/g)	log C_e	log (X/M)
Oil	5.21×10^{-2}	3.18×10^{-2}	−1.283	−1.497
	1.23×10^{-2}	6.42×10^{-3}	−1.910	−2.192
	6.35×10^{-3}	4.00×10^{-3}	−2.197	−2.398
	1.06×10^{-3}	6.68×10^{-4}	−2.976	−3.176
	5.51×10^{-4}	2.65×10^{-4}	−3.259	−3.577
	5.42×10^{-5}	3.01×10^{-5}	−4.266	−4.522
	9.75×10^{-6}	6.29×10^{-6}	−5.011	−5.201
Hexane	1.35×10^{-1}	4.32×10^{-2}	−0.869	−1.365
	2.68×10^{-2}	7.56×10^{-3}	−1.572	−2.121
	1.20×10^{-2}	3.26×10^{-3}	−1.922	−2.487
	2.36×10^{-3}	3.73×10^{-4}	−2.625	−3.428
	1.04×10^{-3}	3.16×10^{-4}	−2.981	−3.500
	8.59×10^{-5}	1.66×10^{-5}	−4.066	−4.779
	6.48×10^{-6}	2.22×10^{-6}	−5.188	−5.654
Water	9.20×10^{-5}	1.31×10^{-3}	−4.036	−2.882
	3.48×10^{-5}	5.74×10^{-4}	−4.458	−3.241
	1.90×10^{-5}	2.94×10^{-4}	−4.722	−3.532
	8.50×10^{-6}	1.43×10^{-4}	−5.071	−3.844
	8.45×10^{-6}	1.43×10^{-4}	−5.073	−3.844
	2.54×10^{-6}	2.73×10^{-5}	−5.594	−4.561

Table 3—Initial concentration of monomer in polymer corresponding to allowed equilibrium concentration in specific extractants

Allowed equilibrium (C_e) conc in liquid phase g/g		W_o/M (g/g)		
		Water	Hexane	Oil (food)
Unplasticized resin	1×10^{-9}	889×10^{-9}	30×10^{-9}	40×10^{-9}
	1×10^{-6}	128×10^{-6}	15×10^{-6}	18×10^{-6}
Plasticized resin	1×10^{-9}	21×10^{-9}	10.3×10^{-9}	17.3×10^{-9}
	1×10^{-6}	26×10^{-6}	10.3×10^{-6}	10.6×10^{-6}

(5) or, $W_p = \frac{\alpha C M W_s}{S}$ where $W_s \rightarrow O$ and S has values of "good" solvents

V = actual volume occupied by monomer at equilibrium

V_M = total volume available for sorption.

We can solve either of these equations for A—concentration of monomer in extraction phase at "tolerance" level or A = $W_s/MR = C_e$ and for W_o/M = the "allowable" initial residue in the polymer e.g., by Freundlich, from Eq (2).

(6) $\frac{W_o}{M} = (KA)^{1/n} + AR$, by Langmuir and Eq. (5)

(7A) $\frac{W_o}{M} = \frac{\alpha CAMR}{S} + AR$, but since S = (S) X MR, = W_{sat}

(7B) $\frac{W_o}{M} = \frac{\alpha CA}{(S)} + AR$

From the identity of the two isotherms,

(8A) $(KA)^{1/n} = \frac{\alpha CA}{(S)}$

(8B) $K = \frac{\alpha C n}{(S)} A^{n-1}$

Thus α and C can be calculated from the Langmuirian plots to compare with Freundlich K and n using experimental data relating W_o, M, R, C_e and (S).

It should be noted that when n = 1, $K = RW_p/W_s = \alpha C/(S)$ or K is the partition coefficient for a linear desorption or partition of a solute between two ideal solvents showing activity coefficient of a.

Where $n > 1$, K is a function of the concentration of migrant and not a constant giving nonlinear desorption isotherms.

Sorption isotherms of this type are produced when strong interactions occur between sorbant and solute in a monomolecular layer involving "active sites." This condition should also hold for migration or desorption where solute/solvent interactions are weaker than solute/polymer ones e.g., in solvent-food systems which have poor solubility for the migrant, as in the case of vinyl chloride-water.

Eq. (6) has been used to provide the values of initial monomer content corresponding to selected equilibrium concentration of migrant in the polymer contacting phase. The calculated values are given in Table 3.

Thus the hypothesis has been confirmed by the present data for unplasticized resins that vinyl chloride and other such polar migrants will show an "Essentially Zero" migration at finite low concentration.

For plasticized resins, the partition constants between resin and air with the gas phase as an infinite sink (particularly at an elevated temperature), the levels of retained monomer can be reduced to the ppb level (Berens, 1974). Here, also, low resin mass as in films are used which also restricts total migrant mass.

Desorption studies are being extended with further partition studies at lower concentrations and analytical sensitivities with actual as well as simulated food systems.

Studies on possible biochemical reactions of VCM with tissue enzymes could be used to test the first postulate. A rational basis would then be provided for the definition of a "no migration level" for safety. Such a basis should be more satisfactory than the present shifting base of lowest limit of analytical detection which offers neither the assurance of biochemical safety nor a basis for evaluation of the economics of manufacturing processes.

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Ms received 9/13/75; revised 11/29/75; accepted 12/2/75.

A paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers—The State University of New Jersey, Food Science Department, Cook College, P.O. Box 231, New Brunswick, NJ 08903.

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A Research Note
POLYPHENOLIC COMPOUNDS IN THE PULP OF Mangrifera indica L.

ABSTRACT

Polyphenolic compounds were isolated from the pulp of three varieties of mangoes by ethyl acetate extraction. The compounds were separated by two-dimensional paper chromatography. The individual compounds were identified by R_f values, fluorescent behavior and degradation products. Gallotannin, m-digallic acid, gallic acid, unknown hydrolyzable tannin and mangiferin were identified. Phenolic compounds which are known to be responsible for astringency and browning in fruits were not detected by this extraction procedure.

INTRODUCTION

POLYPHENOLIC COMPOUNDS play an important role in the biological functions, metabolism, and respiration of plants. In fruits they are related to astringent flavor and enzymic browning (Swain, 1962). Their identification has been the subject of many studies.

Hulme (1971) stated that polyphenolic compounds must make an important contribution, because of the astringency of tannins, to the flavor of the mango as they do to other fruits. He reported that the nature of the compounds contributing to the "tannin" content have not been fully investigated.

In this work, polyphenolic compounds in mango fruit were separated by solvent extraction and purified by two-dimensional paper chromatography. R_f values obtained from various solvent systems were used to identify the compounds.

MATERIALS & METHODS

FRUITS of three mango cultivars – Alphonso, Kitchener and Abu Samaka – were harvested during the peak of the seasons (1971/72 and 1972/73) and ripened in the laboratory (Avg temp 28–30°C, relative humidity 45%). 150 fruits were peeled and sliced at 0°C and the slices mixed together thoroughly, flushed with nitrogen gas and kept at 20°C. Isolation of polyphenolic compounds was accomplished by extraction of the mango pulp with water, 95% ethanol and acetone in a Waring Blendor.

The polyphenolic compounds in each solvent extract were re-extracted by ethyl acetate as described by El Sayed and Luh (1965). The compounds were separated by two-dimensional paper chromatography with n-butanol, acetic acid, water (4:1:5) and 2% acetic acid and with tertiary amyl alcohol, acetic acid, water (6:0.1:94) and water saturated secondary butanol. The individual compounds were identified by their R_f values, fluorescent behavior, color reactions, degradation products (spots 1 and 6) according to El Sayed and Luh (1965) procedure.

RESULTS & DISCUSSION

TABLES 1 AND 2 show the R_f values and color under Ultraviolet light (with and without ammonia vapor) of mango polyphenolic compounds present in the ethyl acetate extract. Spots 1, 1a, 2, 4, 6 and 7 appeared in the chromatogram prepared from water, ethanol and acetone extracts. Spot 8 appeared on samples extracted with ethanol while spot 3 appeared clearly on samples extracted with water or acetone.

Table 1—Two dimensional paper chromatography of polyphenolic compounds in mangoes extracted with ethyl acetate

		R_f value			
Spot no.	Tentative identification	BAW (4:1:5)	2% acetic acid	Tert amyl alcohol HAC/water	Water sat butanol
1	Gallotannin	0.52	0.00	0.80	0.90
1a	m-digallic acid	0.52	0.07	0.12	0.24
2	Unknown + carotenoids	0.81	0.00	0.00	0.89
3	Mangiferin	0.51	0.21	0.19	0.30
4	Gallic acid	0.58	0.38	0.34	0.58
6	Gallic acid + glucose (unknown hydrolyzable tannin)	0.55	0.63	0.44	0.43
7	Unknown	0.37	0.68	0.63	0.28
8	Unknown	0.40	0.83	0.55	0.16
Authentic compounds					
	Gallotannin	0.52	0.00	0.80	0.90
	m-digallic acid	0.52	0.06	0.12	0.24
	Mangiferin	0.50	0.20	0.20	0.31
	Gallic acid	0.60	0.40	0.31	0.55

Table 2—Color reactions of polyphenolic compounds in mango fruits

Spot No.	Tentative identification	Fluorescence under UV light	Visible light	UV light + NH_3	$FeCl_3$ $K_3Fe(CN)_6$	Vanillin HCl
1	Gallotannin	–	–	–	blue	–
1a	m-digallic acid	–	–	–	blue	–
2	Unknown	blue	–	blue	blue	–
3	Mangiferin	v. faint orange	–	v. faint yellow	faint blue	–
4	Gallic acid	–		–	blue	–
6	Gallic acid + glucose (unknown hydrolyzable tannin)	–	–	–	blue	–
7	Unknown	–	–	–	blue	–
8	Unknown	–	–	–	blue	–
Authentic compound						
	Gallotannin	–	–	–	blue	–
	m-digallic acid	–	–	–	blue	–
	Gallic acid	–	–	–	blue	–
	Mangerin	Orange	–	Yellow	blue	–

The three mango cultivars showed the same pattern of spots. Spots 5, 9, 10 and 11 were fluorescent only under UV but did not give blue color with $FeCl_3 - K_3Fe(CN)_6$ reagent and thus were not considered polyphenolic compounds.

Authentic polyphenolic compounds were run individually and concurrently with the extract on paper. Gallotannin (Spot 1), m-digallic acid (Spot 1a), mangiferin (Spot 3) and gallic acid (Spot 4) were tentatively identified in the extract. The compounds were further characterized by acid hydrolysis products and by their different positions in paper using different solvents.

Spots No. 1 and 6, after being hydrolyzed and tested, gave rise to 2 spots which were shown to be glucose and gallic acid as judged from the R_f values and color reactions of glucose and gallic acid. From tests with authentic gallotannin, and hydrolysis, spot 1 was confirmed to be a gallotannin. Spot 6 could be identified as a hydrolyzable tannin containing gallic acid and glucose. Further identification of this spot is needed.

The results obtained indicate that the majority of the polyphenolic compounds detected in ethyl acetate extracts of mango fruits were hydrolyzable tannins. Condensed tannins like catechins and leucoanthocyanins, which are responsible for astringency and browning in fruits, were not detected in ethyl acetate extract from mangoes. Other flavonoid compounds like rutin, quercetin, etc. were not detected by the methods used in this investigation. Chlorogenic acid, and caffeic acid which are the precursors of the substance responsible for the enzymic browning of fruits were not detected in these mango fruits.

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Ms received 10/6/75; revised 1/16/76; accepted 1/20/76.

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A Research Note
CASHEW PHOSPHOLIPIDS

ABSTRACT

Phospholipids were isolated by silicic acid column chromatography (CC) from cashew nuts and cashew apple juice. Component phospholipids were separated by two-dimensional thin-layer chromatography (TLC). Cashew nut oil and cashew apple juice lipids gave 9 and 11 TLC spots, respectively, positive to the specific molybdenum spray. Phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) isolated from cashew nut lipid phospholipids were analyzed for fatty acid composition. The major fatty acids in PE and PC were palmitic, stearic, oleic, and linoleic representing 85.3% of the total in PE and 73.3% in PC.

INTRODUCTION

CASHEW NUT TREE (*Anacardium occidentale* L.) is native to Brazil (Woodroof, 1967). An interesting aspect of the tree is its "false fruit," the swollen peduncle or hypocarp, commonly known as cashew apple. It is pear shaped or rhomboid to ovate, from 6–10 cm long and bright yellow to red color. It has a waxy skin, is juicy with spongy pulp and musklike fragrance. The "apple" is very astringent until fully ripe when it is sub-acid to acid and edible. This "apple" is actually the receptacle for the true fruit, the cashew nut. The cashew apple is noted for its high ascorbic acid content.

Since there is a lack of published information, the purpose of this work was to characterize the phospholipids of cashew apple juice and cashew nut kernel.

EXPERIMENTAL

Cashew material

Shelled, unroasted cashew nuts were obtained from a processing plant in Fortaleza, Ceara, Brazil.

The cashew apple juice was prepared in a food processing plant in Ceara, Brazil. Juice preparation involved extraction, deaeration (75°C for 2 min at 600 mm Hg), homogenization (100 atm), heat treatment (98°C for 96 sec), packaging in clear glass containers and cooling to 26°C.

The cashew material was shipped to the laboratory via airmail.

Solvents

Reagent grade solvents were redistilled in an all-glass unit to nano-grade purity.

Lipid extraction

Lipid extraction from juice and nut material was by the method of Bligh and Dyer (1959). Chloroform solvent was evaporated to a small volume (about 5 ml) under reduced pressure at 45°C, and the lipid stored in sealed containers under nitrogen atmosphere at 0°C until analyzed (ca 72 hr).

Lipid fractionation

Extracted lipids were separated by silicic acid column chromatography according to the method of Rouser et al. (1967). No detectable levels of phosphorus were found in the neutral lipid and glycolipid fractions based on the specific reaction with molybdenum blue spray.

Phospholipids separation

Phospholipids were separated by a two-dimensional TLC procedure (Parsons and Patton, 1967) using a solvent system consisting of chloroform-methanol-water-28% aqueous ammonium hydroxide (65:35:4:0.5 v/v) in the first dimension and chloroform-acetone-methanol-acetic acid-water (50:20:10:10:5 v/v) in the second dimension.

After removal from the chamber, the plates were air dried (20°C). Detection methods involved spraying the plates with the following reagents (Applied Science Labs., Inc.):

1. Ninhydrin–for phospholipids containing free amino groups such as phosphatidyl ethanolamine (PE) and phosphatidyl serine (PS). The spots which showed positive reaction to this spray gave a purple color.
2. Rhodamine 6 G–lipids gave fluorescence under long wave UV light.
3. 2′,7′-dichlorofluorescein–gave fluorescence with saturated and unsaturated lipids.
4. Molybdenum blue reagent–specific for phospholipids.

For the detection of phospholipids whose fatty acids were to be analyzed by GLC, the plates were sprayed with rhodamine 6 G or with 2′,7′-dichlorofluorescein and inspected under UV light. The observed spots were then outlined with a needle point.

Successive sprays were used in some plates in the following way: the plates were first sprayed with rhodamine 6 G and the spots marked. After the plate was completely dried, it was sprayed with ninhydrin and the spots which gave positive reaction were noted. Finally, the plate was treated with molybdenum spray. Individual phospholipids were identified by means of these selective spray reagents and by comparing Rf values with those of pure reference compounds. All reference phospholipids were obtained from Applied Science Labs., Inc.

Quantitative analysis of phospholipids

Quantitative analysis of phospholipids was made by an adaptation of the method of Rouser et al. (1966).

The relative amount of phosphorus in each spot was determined by using reagents supplied in a kit (Pierce Chemical Co.).

Preparation of phospholipid fatty acid methyl esters

After TLC separation, the plates were sprayed with rhodamine 6 G. The two major spots were then scraped from the plate and eluted with chloroform:methanol (1:1) 50 ml. The solvent was evaporated to a small volume under a stream of nitrogen and kept in small vials at −10°C. Fatty acid methyl esters for GLC analysis were prepared by the method of Metcalfe et al., 1966.

GLC analysis

Samples were injected into a Micro Tek (Micro Tek Instruments, Inc.) gas chromatograph model DSS 170 equipped with a dual flame ionization detector, two glass columns, (dimensions 0.4 cm internal diam × 1.65m long) packed with 15% diethyl glycol succinate (DEGS) on 60–80 mesh Chromasorb W. Carrier gas used was argon at 0.281 gk/cm² (flow-rate – 60 ml/min). Column temperature was 175°C and sample size approx 0.5 microliter. Ester identification was made by comparison with standard compounds injected under the same conditions. Relative amounts of each ester were determined by peak areas comparisons as calculated by an Infrotonic Model CRS-108 integrator (Infrotonic Corp., Houston, Texas).

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Table 1—Relative percentages of phospholipids isolated from unroasted cashew nut

Spot	Component	Proportion of Total (%)
1	LPC	5.1
2	PS	6.5
3	PC	53.6
4	PE	14.0
5	X_1	3.0
6	X_2	3.8
7	X_3	5.0
8	X_4	4.0
9	X_5	5.0

Table 2—Major component fatty acids in phosphatidyl ethanolamine and phosphatidyl choline isolated from unroasted cashew nut oil

Fatty acid	PE (%)	PC (%)
$C_{12:0}$	0.6	2.1
$C_{14:0}$	1.7	6.2
Unknown	1.4	4.6
$C_{16:0}$	16.7	15.8
$C_{16:1}$	2.9	2.4
$C_{16:2}$	0.2	0.4
$C_{18:0}$ iso	2.6	2.3
$C_{18:0}$	3.5	5.5
$C_{18:1}$	57.0	49.5
$C_{18:2}$	11.5	8.0
$C_{18:3}$	1.8	2.3

RESULTS & DISCUSSION

Phospholipids

A two-dimensional chromatogram of phospholipid separation from unroasted cashew nuts gave nine components (Table 1). Unknown phospholipids represented by X_1, X_2, X_3, X_4 and X_5 were positive to the specific molybdenum spray. Four phospholipids were identified as lysophosphatidyl choline (LPC), phosphatidylcholine (PC), PE and PS. These spots showed behavior similar to the standards. PS and PE were also positive to ninhydrin spray. On the basis of other similar work, the spots 5, 6, and 8 could be tentatively identified as phosphatidyl glycerols (Parsons and Patton, 1967). A similar distribution of phospholipids has been found in cocoa bean phospholipids. The major phospholipids in unroasted cashew nut are PC and PE which together contributed over 60% of the total (Table 1).

For further identification, spot number 3 was scraped from the plate and eluted with chloroform-methanol (1:1) and evaporated to small volume. An aliquot was spotted on a plate and developed in one direction with chloroform-methanol-7N NH_4OH. On the same plate, a PE standard was applied.

PC and PE have been found to be the major phospholipids in potato tubers and apples (Galliard, 1968a, b), in cucumbers and peppers (Kinsella, 1971) and in oranges, lemons and grapefruit (Vandercook et al., 1970).

A typical two-dimensional chromatogram of cashew apple juice phospholipids gave eleven spots positive to molybdenum spray. The following spots were identified: LPC, PC and PE. Three others were identified as phosphatidyl glycerols (Galliard, 1968a, b). The phospholipids from cashew apple juice were not quantified due to the limited material available.

Phospholipid fatty acids

The major fatty acids in cashew PE and PC are palmitic, oleic and linoleic (Table 2). These fatty acids together represent 85.3% of the total in PE and 73.3% of the total in PC. Some differences in fatty acid distribution of these two phospholipids can be observed. PC shows greater percentages of $C_{12:0}$, $C_{14:0}$ and $C_{18:0}$. PE shows greater values for $C_{16:0}$, $C_{18:1}$ and $C_{18:2}$. PE is 73.5% unsaturated and PC 62.6%. Comparable values have been found in orange juice phospholipids (Nagy and Nordby, 1970).

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Ms received 12/13/75; revised 1/29/76; accepted 2/4/76.

Arizona Agricultural Experiment Station Technical Paper No. 2349. Supported in part by Grant AID/1a-145 (Brazil Contract).

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A Research Note

MINERAL COMPOSITION OF PECAN NUTMEATS

ABSTRACT

Sixteen minerals were quantitated in the nutmeats of 10 pecan cultivars (*Carya illinoensis* Wang) with atomic absorption and emission spectroscopy. Significant difference ($P < 0.01$) existed among cultivars in the quantities of Cu, Fe, Cr, Mn, B, Zn, Ba, P, K and Ca. Differences were not significant at the 5% level in the quantities of Co, Mo, Sr, Na, Al and Mg.

INTRODUCTION

THE ADVERSE EFFECT of many metals on vegetable oils has long been recognized. Among the more deleterious are Cu, Fe, Co and Cr that accelerate the oxidation of fatty acids and the appearance of reversion flavors at low levels of oxidation (Lundberg, 1962). Zn, Pb, Ca and Mg are also implicated, but to a lesser extent than the preceeding 4 elements. Lundberg showed that 0.1 ppm Fe, 0.01 ppm Cu, and 3 ppm Co were deleterious to the flavor of soybean oil, and that the "native" Fe and Cu contents of this oil were significant in flavor stability.

Although these metals decrease the stability of vegetable oils and other high fat commodities, many minerals are essential for normal metabolic functions and are required components in a balanced diet. Ca, P, K, Na, Mn and S are required in fairly large quantities and Fe, Cu, Co, Mg, Zn, I and Mo in micro quantities (Pike and Brown, 1967). There is also evidence that Se and Cr function in metabolic systems (Pike and Brown, 1967; Watt and Murphy, 1970) and their content in foods is a growing concern of nutritionists. V, Ni, and Sn also might be essential for man, having been shown to be essential for laboratory animals (Nielsen, 1974).

The ability of plants to assimilate trace minerals varies significantly among cultivars within species (Kleese et al., 1968; Gorsline et al., 1964; Peterson et al., 1975) and within cultivars as a result of environment (Kleese et al., 1968; Gammon and Lam, 1974). This effect of environment might explain variations among cultivars in susceptibility of component lipids to become rancid, and could provide a basis for improved stability of stored products through genetic manipulation and/or improved environmental control.

The present study was conducted to determine the mineral content of pecan nutmeats. Information derived will supplement present data on the nutritional quality of pecans (USDA, 1963), and by comparing the quantities of pro-oxidant metals in different cultivars grown under similar conditions, provide a basis for explaining variation among cultivars in susceptibility of nutmeats to become rancid.

EXPERIMENTAL

IN-SHELL SAMPLES of pecans (*Carya illinoensis* Wang) were obtained from the U.S. Pecan Field Station, Brownwood, Texas, shortly after the 1974 harvest. Samples were taken from standard (Mahan, Desirable, Stuart) and newly developed (Cheyenne, Western, Tejas, Cherokee, Shoshoni, Schley × Barton, Schley × McCulley) cultivars. Ten-lb (4.5 kg) samples of each cultivar were taken from mature trees that were free from disease and disorders and had been grown on similar soils. Each cultivar had received similar levels of fertilization and sprays for insects and disease control. In-shell samples were stored in polyethylene bags at −35°C until analyzed.

Fifty fully developed nuts from each cultivar were selected for analysis. These samples were hand shelled and the nutmeats from each cultivar (ca 200g) were combined and chopped with a standard food chopper. Samples for mineral analysis and moisture determinations were taken from these composites.

Moisture was determined on duplicate 5-g samples from each cultivar taken from nutmeat particles passing a #14 screen (1.41 mm). The samples were dried to constant weight at ambient temperature over P_2O_5 in an evacuated desiccator (ca 18 hr).

Minerals were determined by the Soil Testing and Plant Analysis Laboratory, University of Georgia, Athens, by the procedures of Isaac and Johnson (1975). Cu, Fe, Co and Cr determinations were made on $HClO_4$ digested samples with a Perkin-Elmer model 403 atomic absorption spectrophotometer. Assays of the remaining minerals were made with a Jarnell-Ash model 82-500 direct reading emission spectrograph on samples that had been dry ashed at 500°C for 2 hr followed by addition of HNO_3 and further ashing for 1 hr at 500°C. Assays were made in duplicate and results reported as ppm Cu, Fe, Co, Cr, Al, Mn, B, Zn, Mo, Sr, Ba, Na and percent P, K, Ca and Mg.

Variation among cultivars was determined by analyses of variance with a model 700 Wang programmable calculator. Means of analyses were then determined, converted to mg/100g of dry nutmeat and separated by Duncan's multiple range and multiple F tests (Duncan, 1955).

RESULTS

RESULTS of these analyses are presented in Table 1. Average moisture content of the 10 cultivars was 4.7%, range 3.0–5.8%. F values were significant ($P < 0.01$) among cultivars in the quantities of Cu, Fe, Cr, Mn, B, Zn, Ba, P, K and Ca. Means for these elements were 1.08, 2.20, 1.20, 3.28, 0.62, 7.02, 0.56, 450, 460 and 5.8 mg/100g of dry nutmeat, respectively. Contents of Mo, Sr and Mg were 0.06, 0.58 and 140 mg/100g, respectively, and did not differ significantly ($P > 0.05$) among cultivars. Traces (< 1 ppm) of Co were present in all cultivars. Varying amounts of Ca were in all cultivars except Cheyenne, Cherokee and Stuart. Significant differences ($P > 0.05$) between replicate analyses were found in the Na assays. Values for Na reported in Table 1 are therefore not the means of duplicate analyses but are values from one assay chosen for conformance with published data (Heinz, 1956).

DISCUSSION

SIXTEEN MINERALS were identified and quantitated in the nutmeats of 10 pecan cultivars. These data supplement published data (USDA, 1963; Heinz, 1956) on the nutritional quality of pecans and provide information that will aid in the collation of data for the Nutrient Data Bank. This study showed that pecan nutmeats contain pro-oxidant metals in

Table 1—Minerals of pecan nutmeats (Mean concentrations, mg/100g[a])

	% Moisture	Cu	Fe	Co[b]	Cr	Al	Mn	B	Zn	Mo	Sr	Ba	Na	P	K	Ca	Mg
Cheyenne	3.0	1.44a	1.93f	tr.	0.11d	0	2.42de	0.57de	5.60cd	0.07	0.52	0.67bc	C.0	390bc	330f	0	140
Western	5.3	1.22b	2.52b	tr	0.15b	0	4.39ab	0.42ef	8.21b	0.0E	0.53	0.63cd	C.63	430bc	370ef	5.3c	130
Tejas	5.3	1.22b	2.65a	tr	0.13c	0	1.85de	0.90a	7.18bc	0.0E	0.74	0.90a	C.21	470bc	440cde	5.3c	160
Cherokee	5.3	1.10c	2.41c	tr	0.20a	0	1.73e	0.74bc	8.03b	0.07	0.64	0.32ef	C.84	430bc	540b	0	150
Schley X Barton	4.6	1.09cd	2.15e	tr	0.13c	0	4.83ab	0.63cd	5.30d	0.07	0.52	0.47de	0.21	440bc	390def	5.3c	150
Shoshoni	3.4	1.06d	2.28d	tr	0.16b	0	3.11cd	0.52de	6.26cd	0.0E	0.57	0.41ef	0.62	500ab	490bc	5.2c	160
Stuart	4.9	1.08cd	2.02f	tr	0.16b	0	2.21de	0.63cd	8.16b	0.0E	0.58	0.48de	0.0	470bc	470bcd	0	120
Schley X McCulley	4.3	0.90e	2.00f	tr	0.15b	0	5.33a	0.63cd	5.65cd	0.08	0.52	0.63cd	0.84	400bc	430cde	10.5b	120
Mahan	5.5	0.87f	2.11e	tr	0	0	2.97cde	0.32f	5.40d	0.07	0.53	0.27f	0.63	340c	440cde	5.3c	170
Desirable	5.8	0.82g	1.93f	tr	0	0	3.99bc	0.80ab	10.40a	0.05	0.69	0.80ab	0.42	610a	660a	21.2a	170
Mean Value	4.7	1.08	2.20	tr	1.20	0	3.28	0.62	7.02	0.0E	0.58	0.56	0.44	450	460	5.8	140

[a] Dry-weight basis
[b] Tr = < 1 ppm
[c-g] Values within a column followed by the same letter or letters do not differ significantly at the 5% level.

quantities that would be important in storage stability. In addition, the significant variation in minerals among cultivars provides a plausible explanation for the variation in susceptibility of stored nutmeats from certain cultivars to degradation by oxidative rancidity.

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Appreciation is expressed to Dr. R.A. Isaac and Mr. W.C. Johnson for cooperation in these analyses.

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A Research Note

EMULSION CAPACITY AND FOAM STABILITY OF RE-EXTRACTED SUNFLOWER MEAL (Helianthus annuus)

ABSTRACT

Emulsification and foam stabilization attributes of residual lipids in hexane-extracted sunflower meal were determined. Re-extraction with various solvents did not markedly affect emulsifying capacity. Methanol washing increased foam stability.

INTRODUCTION

EMULSIFYING and foaming properties of solvent-extracted sunflower meal have been reported by Lin and Humbert (1974) and by Huffman et al. (1975). The ability of nonprotein constituents to aid in the formation of emulsions and foams remains unknown.

Sunflower meal, containing 35% less protein than its protein isolate, has the same whippability. Constituents other than protein aid in formation of whipped foams (Lin and Humbert, 1974). Lawhon et al. (1972) reported that the water-soluble extract of many oilseed meals, including sunflower meal, has a high whipping potential.

In this investigation, hexane-extracted sunflower meal was subsequently extracted using several solvents. Extractability of residual lipids and emulsifying and foaming properties of the resultant meals were determined.

EXPERIMENTAL

MEAL PREPARED from kernels of the Peredovik cultivar was extracted with hexane (Wu, 1975). Subsequent solvent extraction of the meal involved: benzene, petroleum ether, chloroform, methanol, chloroform/methanol (2:1 v/v) and ethanol/ether/water (2:2:1 v/v). Solvent extracts were used for the determination of the total weight and lipid loss during solvent extraction and the estimation of the amount of residual lipids retained after solvent-extraction.

To determine loss of total weight, the solvent odor was dissipated and the extract was weighed at 2-hr intervals to constant weight. Moisture content was determined by the air-oven method (AOAC, 1965). Lipid loss was measured by redissolving the extracts in original solvents and quantifying by hydroxylamine hydrochloride colorimetric method proposed by Hill (1946, 1947). Residual lipids in the meal were determined using the hydrolytic method of Wren and Wojtczak (1964). The emulsifying capacity was measured by the red-0 dye emulsion inversion method of Marshall et al. (1975). Foam suspensions were created by dispersing meal at a ratio of 3/100 in pH 7 phosphate buffer. The suspension was whipped for 6 min at ambient temperature with a Sunbeam Mixmaster set at speed 12 (Huffman, 1974). The liquid volume released by the foam in 1 hr was considered indicative of foam stability.

RESULTS & DISCUSSION

RE-EXTRACTION with methanol resulted in the largest loss of total weight from hexane-extracted meal. This was followed by ethanol/ether/water, chloroform/methanol, petroleum ether, benzene, and chloroform (Table 1). In wheat flour Wootton (1966) found chloroform/methanol to be better than ethanol/ether/water.

Table 1—Extractability[a], emulsion and foam properties of sunflower meal.

Solvent	Total wt loss % dry wt	Lipid loss[b] % dry wt	Emulsifying capacity[c] % oil phase vol	Foam stability[d] % increase
Benzene	2.9	0.41	−2.1	+5.3
Chloroform	2.1	0.30	−1.9	+8.7
Methanol	19.5	0.79	−1.6	+15.8
Petroleum Ether	4.0	0.27	−0.3	−3.5
Chloroform/methanol	5.8	0.56	−3.5	+7.0
Ethanol/ether/water	16.0	2.39	−3.3	+1.8

[a] 24 hr re-extraction of hexane defatted meal
[b] Initial lipid content 2.77% dry wt
[c] % oil phase volume at inversion point compared to hexane defatted meal
[d] Compared to hexane extracted meal 1 hr after whipping

Ethanol/ether/water removed the largest amount of lipid; followed by methanol, chloroform/methanol, benzene, petroleum ether, and chloroform (Table 1). This sequence is different from that of the loss of weight. Methanol caused greater weight loss than ethanol/ether/water, but the reverse was true for lipid removal.

The amount of residual lipids in hexane-extracted meal was 2.77%. Residual lipids in re-extracted meals ranged from 0.38% to 2.72% dry weight depending on solvent used. Emulsifying capacity was not markedly affected by the subsequent solvent-extraction.

Foam stability of hexane-extracted meal was related to the liquid released in 1 hr after whipping. With the exception of petroleum ether, re-extraction increased foam stability. This was most pronounced with methanol (Table 1) and similar to the observation on soybean protein reported by Eldridge et al. (1963). They proposed the existence of an alcohol-soluble foam inhibitor.

At the 5% level of significance, residual lipids and functional properties were not correlated.

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Ms received 6/16/75; revised 1/19/76; accepted 1/26/76.

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A Research Note

THE RELATIONSHIP BETWEEN REFRACTIVE INDEX AND SPECIFIC GRAVITY OF AQUEOUS SUCROSE SOLUTIONS

ABSTRACT

Published data on the refractive index and specific gravity of aqueous sucrose solutions were compared. It is shown that small, but significant, systematic errors are present in the published tables. A means for the reduction of these errors is suggested.

INTRODUCTION

THE RELATIONSHIP between the then-accepted tables of the refractive index of aqueous sucrose solutions, and their specific gravities, was discussed in a previous paper (Basker, 1967). Since that time, the International Scale of refractive indexes of sucrose solutions (AOAC, 1965) has been replaced by a new table (Carpenter, 1970; AOAC, 1975). The Domke table of apparent specific gravity of sucrose solutions (AOAC, 1965) has also been dropped, as being "surplus;" the Plato table (AOAC, 1975) was retained in its stead.

The accuracy of the AOAC (1975) tables is now examined, using the linear relationship which has been shown to exist between these two parameters for solutions other than those of strong electrolytes (Glover and Goulden, 1963; Basker, 1969a, b). A comparison is also made with the density data reported by Thiele (1962).

METHODS

THE REGRESSION LINE of the refractive index of sucrose solutions on the concentration in weight-to-volume (W/V) units was calculated using the data of the AOAC (1975) tables. The specific gravities at 20/20°C were employed. The refractive index at zero concentration (i.e., water) is known with high degree of accuracy (Weast, 1971), and the regression was therefore calculated through this point (Brownlee, 1965).

The specific gravity data reported by Thiele (1962) are given at 20/4°C, and were adjusted to 20/20°C in order to be comparative. The regression line was calculated as before.

RESULTS & DISCUSSION

EVEN WITH THE AOAC (1965) tables, the data were at first sight almost perfectly compatible, with a correlation coefficient only fractionally less than unity (Basker, 1967). The regression of the refractive index on concentration by volume, with the AOAC (1975) tables, was found to be:

$$\text{Refractive index} = 1.3330 + 0.001402 \times \text{\% sucrose (W/W)} \times \text{specific gravity} \quad (1)$$

The differences between the tabulated data and those predicted from Eq (1) were calculated, and are shown as line A in Figure 1; their magnitude (tabulated minus predicted) ranges to +0.0008 at 40–50% sucrose (W/W), (equivalent to about 0.4% sucrose), and to −0.0016 at 86% sucrose (W/W) (equivalent to about 0.6% sucrose). It is clear from Figure 1 that the differences are systematic. Ideally, the differences would be randomly distributed with insignificant variation around the zero line. The residual standard deviation was 0.0006 refractive index units. Because the tables are compared only with each other, further information is required to determine whether the systematic errors found lie in the refractive index table or in the specific gravity table, or in both.

The regression line of the refractive index (AOAC, 1975) on the adjusted concentration by volume (Thiele, 1962) was found to be:

$$\text{Refractive index} = 1.3330 + 0.001410 \times \text{\% sucrose (W/W)} \times \text{specific gravity} \quad (2)$$

The differences between the tabulated data and those predicted from Eq (2) were calculated, and are shown as line B in Figure 1; their magnitude (tabulated minus predicted) ranges to +0.0004 at 30–40% sucrose (W/W) (equivalent to about 0.2% sucrose), and to −0.0010 at 71% sucrose (equivalent to about 0.4% sucrose). The residual standard deviation was 0.0003 refractive index units, an improvement over Eq (1), but the general shape of the curve follows that of line A (see Fig. 1). It follows that at least part of the systematic error lies in the refractive index table.

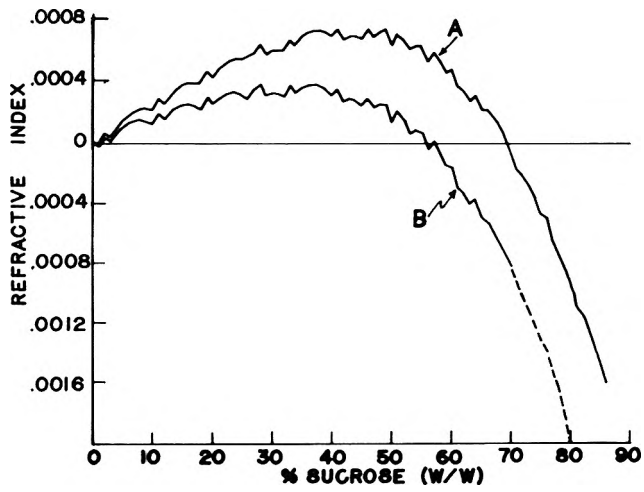

Fig. 1—Differences between tabulated and predicted values of the refractive index of sucrose solutions. (A) Carpenter – Plato data; (B) Carpenter – Thiele data.

CONCLUSIONS

SMALL systematic errors were found in the tables of refractive index and specific gravity of aqueous sucrose solutions (AOAC, 1975). It follows that the data on which the refractive index tables are based (Carpenter, 1970) are not uniformly reliable. The reduction found in the residual standard deviation when independent values of the specific gravity (Thiele, 1962) were employed, indicates that part of the error lies in the specific gravity table (AOAC, 1975).

Correction of both tables in accordance with the best measured data is feasible, if the two parameters are considered together.

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Ms received 10/20/75; accepted 12/8/75.

I thank Dr. M. Shimshoni of the Dept. of Applied Mathematics of the Weizmann Institute of Science (Rehovot, Israel), and Dr. J. Putter of the Div. of Statistics and Experiment Design of the Agricultural Research Organization (Bet Dagan, Israel), for their most helpful advice.

Contribution from the Agricultural Research Organization, The volcani Center, Bet Dagan, Israel, 1975 Series, No. 246-E.

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A Research Note

NITRITE BINDING SITES ON MYOGLOBIN

ABSTRACT

^{15}N-labelled nitrite was used to determine the amount of nitric oxide bound to unheated compared to heated myoglobin. Heated samples contained twice the amount of ^{15}N as unheated samples. The globin portion was likely detached from the myoglobin by heating, and two sites for binding of nitric oxide were therefore made available.

INTRODUCTION

NITRITE added to fresh meat oxidizes meat pigments to the ferric state and produces a greyish brown color. Nitric oxide is formed from nitrite by reductants in the meat, and it is subsequently complexed with oxidized pigments, mainly metmyoglobin. Then, nitrosylmetmyoglobin (brown) is converted to nitrosylmyoglobin (red), and ultimately nitrosylhemochrome (pink) is produced by heating (Fox and Thomson, 1963).

It has been postulated, based on the correlation of the position of the optical absorption maxima to the bond type in iron porphyrin compounds, that the cooked, cured meat pigment is a dinitrosylhemochrome (Tarladgis, 1962). This led to the hypothesis that in the cooked cured pigment, the globin portion becomes detached and both of the coordination positions of the iron are occupied by nitric oxide. However, there has still been some question as to the identity of the final cooked cured pigment (Clydesdale and Francis, 1971; Fox, 1966).

If, in fact, heating detaches the globin portion from the myoglobin in cured meat, the amount of ^{15}N complexed to the pigment must be doubled on cooking after adding ^{15}N-labelled nitrite. Thus an experiment was designed to elucidate the amount of ^{15}N complexed to both the uncooked and cooked pigment.

MATERIALS & METHODS

Material

Sodium nitrite labelled with ^{15}N (96.1% enrichment) was purchased from Prochem., Lincoln Park, N.Y. Equine skeletal muscle myoglobin and sodium ascorbate were obtained from Sigma Chemical Company.

Buffer preparation

1 liter of the buffer solution contained 23.3 mmoles of citric acid and 53.4 mmoles of disodium phosphate to make a pH 5.2 solution.

Sample preparation

Ascorbate to myoglobin and nitrite to myoglobin were both 100:1 molar ratios. These conditions were employed to produce a rapid formation of nitrosylmyoglobin (Fox and Thomson, 1963). 860 mg of myoglobin was dissolved in a 100 ml volumetric flask containing 80 ml of the buffer solution. The solution was prepared with the assumption that the protein contained no water. 5 mmoles of ^{15}N-labelled sodium nitrite and 5 mmoles of sodium ascorbate were added to the myoglobin solution, and the solution was made up to 100 ml. Therefore, the final concentration of the model system was 0.5 mM myoglobin, 50 mM sodium ascorbate and 50 mM sodium nitrite. The system was incubated at 22°C in a dark room.

In order to check the formation of nitrosylmyoglobin, 1 ml of sample was diluted to 10 ml with buffer solution at intervals and the optical density was swiftly monitored in a 1 cm light path curvette with a Gilford 240 Spectrophotometer at 545 nm. After maximum formation of nitrosylmyoglobin (2 hr) the system was divided into two groups for unheated and heated samples.

For the unheated cured system, four samples of 5 ml solution each were separately passed through a Sephadex G-25 column with the elution buffer to remove the labelled nitrite which was not reacted with myoglobin. Thus the nitrosylmyoglobin solution recovered would contain only ^{15}N incorporated into the protein. 25 mmoles of sodium ascorbate was added to 1 liter of the buffer solution to make the elution buffer. Sodium ascorbate was added to maintain nitrosylmyoglobin in the reduced form since the oxidized form is relatively unstable. The column was 20 mm in diam and 300 mm in height. The fraction size was < ml.

Four samples of 5 ml solution after 2 hr incubation were heated in Kjeldahl flasks at 100°C for 10 min to produce the cooked cured system. The samples were rapidly cooled to room temperature following heating. An additional 0.25 mmoles sodium ascorbate was put into each sample to insure reducing conditions for formation of nitric oxide, and the samples were incubated for 2 hr. After incubation, samples were filtered through Whatman Filter Paper No. 50 in Büchner funnels and the cooked cured pigments were collected. The samples were washed four times with the elution buffer to remove unreacted ^{15}N-labelled nitrite. Both the unheated and heated systems were transferred to Kjeldahl flasks for total nitrogen analysis.

Total nitrogen analysis

It is recognized that some forms of nitrogen are difficult to convert to ammonia, and for oxides of nitrogen, a reduction step is often

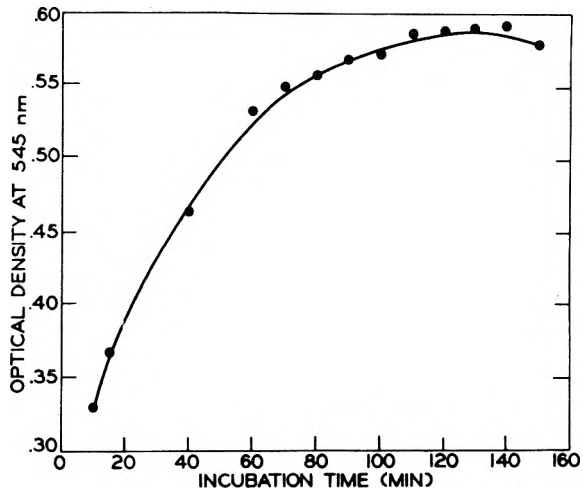

Fig. 1—Formation of nitrosylmyoglobin. The change with time in optical density during incubation of nitrite with myoglobin is plotted.

Table 1—Total nitrogen analysis

Sample	Uncooked	Cooked
1	6.43[a]	6.85
2	5.96	6.50
3	6.25	6.30
4	5.96	6.28

[a] Expressed as mg of nitrogen

Table 2—Analysis for ^{15}N

Sample	Uncooked	Cooked
1	0.0216[a]	0.0430
2	0.0210	0.0432
3	0.0215	0.0430
4	0.0212	0.0436

[a] Expressed as mg of ^{15}N

employed. Total nitrogen was determined, therefore, by a combination of the Devarda and Kjeldahl methods (Davisson and Parsons, 1919). Three ml of 0.25N NaOH and approximately 0.2g of Devarda's metal were first added to a micro-Kjeldahl flask which contained a sample. A column filled with glass beads and containing 8 ml of H_2SO_4 was swiftly attached to it and the solution distilled as far as possible (in our work it was heated for 40 min). The acid in the column was then allowed to suck back into the flask and the beads were washed two times with water. After these steps, the Kjeldahl method was employed for total nitrogen (AOAC, 1970).

^{15}N analysis

The ammonium sulfate solution from the Kjeldahl flask was distilled, titrated, then spotted and dried on a filter paper strip. It was then converted to N_2 by hypobromite on a consolidated Nier isotope mass spectrometer for ^{15}N analysis (Burris and Wilson, 1957).

RESULTS & DISCUSSION

THE CHANGE in optical density during the incubation of myoglobin with nitrite is shown in Figure 1. Fox and Thomson (1963) and Walsh and Rose (1956) reported the mMolar extinction coefficient of nitrosylmyoglobin to be 13.3 and 12.2, respectively. Thus, more than 90% of theoretically possible nitrosylmyoglobin was formed during the 2-hr incubation.

Results for total nitrogen analysis are shown in Table 1. Lower values for uncooked samples are probably due to losses in the chromatography procedures.

Results for ^{15}N incorporation are shown in Table 2. Heated samples contained twice the amount of ^{15}N as unheated samples. Therefore, the globin portion was likely detached from the myoglobin by heating and two sites for nitric oxide were made available. This adds strong support to the suggestion of Tarladgis (1962) that the cooked cured meat pigment presents two binding sites for nitric oxide compared to only one site in the unheated pigment. Therefore, the amount of nitrite used for reaction with myoglobin, under usual curing practices, is substantial, especially in muscle of high pigment content. Moreover, the exposure of an additional site on myoglobin by heating may be a factor in the more rapid flavor change which occurs in heated uncured meat compared to heated cured meat.

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Ms received 12/18/75; accepted 2/4/76.

Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison and by the Food Research Institute.

Appreciation is expressed to G. Woolford and M. Greaser for their suggestions and assistance.

Presented at the 36th Annual Meeting of the Institute of Food Technologists, Anaheim, Calif., June 6—9, 1976.

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A Research Note
COMPOSITION OF SOME COMMERCIAL DRY SAUSAGES

ABSTRACT

Moisture content of 8 types of dried sausage products varied from 58.9–26.4%. Fat, ash, protein nitrogen and total acidity values of each type increased as the moisture content decreased. Bacterial counts indicated that the majority of the sausages were fermented, containing the same range of lactic acid bacterial counts as found for total plate counts. Sausage pH ranged from a low of 4.42 to 5.58. A classification system of dried sausages based on moisture-to-protein ratio and percent moisture was found by regression analysis to fit a linear plot. The classification system also included descriptive terminology generally used in industry to denote the stage of product dryness.

INTRODUCTION

DRYING PROCEDURES for all forms of summer or dried sausage products containing pork and processed at less than 137°F are specified by meat inspection regulations (APHIS, 1973). The drying times and temperatures are based primarily on sausage diameter. Not all dry sausages contain pork tissues, but they do follow similar drying procedures. During studies on summer style dry sausages (Acton et al., 1972; Wardlaw et al., 1973; Keller et al., 1974; Acton and Keller, 1974) our laboratory conducted compositional analyses of summer sausages available at the retail level. Due to the variation found in moisture content, the analyses were expanded to include a broader range of products.

The purpose of this report is to demonstrate the use of moisture and protein values of dry and/or dry fermented sausages in a classification system modified from Kramlich's (1971) moisture criteria and Wilson's (1960) descriptive terminology. Additional compositional data were collected to provide a profile of products retailed in eight product categories.

MATERIALS & METHODS

SAMPLES of products generally referred to as semidry and dry sausages were collected at retail meat displays near Clemson, S.C. over a 9-month period. One exception, San Francisco dry salami, was shipped directly to the authors from California and was not available locally. In most cases at least two or three time intervals of several months duration were allowed prior to obtaining a second and third sample. Similar products were chosen from various manufacturers to give a reasonable estimate of the variation in composition and other characteristics found at the retail distribution level.

Analyses of samples were conducted in duplicate at each sampling. Moisture, fat, ash, and salt content were determined by AOAC (1970a, b) procedures. The salt content, expressed as NaCl, was measured by the method of QUANTAB Chloride Titrators. The Kjeldahl nitrogen method (AOAC, 1970a) was used for nitrogen determinations. The total nitrogen values were corrected for nonprotein nitrogen (NPN) to separate protein nitrogen from NPN. NPN was determined by extracting 10-g samples with distilled water and preparing 5% trichloroacetic acid filtrates.

The procedures of Keller et al. (1974) were used to obtain sausage pH and acidity (lactic acid equivalent). Total acidity was calculated as lactic acid. Total bacteria and lactic acid bacteria counts were conducted using 20g samples and plating on standard plate count agar (APHA, 1966) and the V-8 medium of Fabian et al. (1953), respectively.

Table 1—Average composition and characteristics of some commercial dry sausages

Product Type[a]	Moisture (%)	Fat (%)	Ash (%)	Salt (%)	Nitrogen: Protein N (%)	Nitrogen: NPN (%)	pH	Total acidity[b] (%)	Bacterial counts/g sausage[c]: Total	Bacterial counts/g sausage[c]: Lactic
Lebanon bologna (5)	58.92 ± 2.84	15.12 ± 0.72	4.30 ± 0 57	3.53 ± 0.65	2.55 ± 0.23	0.47 ± 0.04	4.42 ± 0.14	1.95 ± 0.25	10^7–10^8	$<10^3$
Thuringer (13)	48.14 ± 1.93	29.77 ± 2.80	3.58 ± 0 43	2.74 ± 0.32	2.26 ± 0.16	0.32 ± 0.04	5.08 ± 0.42	1.20 ± 0.26	10^3–10^7	10^5–10^6
Semidry salami (8)	46.6 ± 4.67	30.24 ± 3.79	4.36 ± 0.40	4.09 ± 0.55	2.40 ± 0.12	0.35 ± 0.10	5.58 ± 0.53	1.01 ± 0.34	10^3–10^4	$<10^2$
Summer sausage (19)	46.34 ± 5.56	31.06 ± 5.61	3.74 ± 0.52	3.15 ± 0.51	2.27 ± 0.15	0.35 ± 0.07	5.09 ± 0.43	1.23 ± 0.36	10^3–10^4	10^4
Genoa salami (8)	36.21 ± 2.57	33.67 ± 1.77	5.84 ± 0.62	4.54 ± 0.54	3.04 ± 0.24	0.58 ± 0.08	4.89 ± 0.25	2.30 ± 0.20	10^3–10^7	10^2–10^6
Dry salami (11)	35.49 ± 3.02	34.04 ± 3.50	5.43 ± 0.68	4.67 ± 0.53	3.02 ± 0.19	0.54 ± 0.05	5.04 ± 0.32	2.15 ± 0.38	10^3–10^6	10^3–10^5
Pepperoni (14)	28.50 ± 3.83	42.96 ± 3.73	5.14 ± 0.49	4.43 ± 0.74	2.91 ± 0.25	0.43 ± 0.04	5.36 ± 0.41	1.31 ± 0.37	10^4–10^7	10^2–10^6
S.F. Dry salami (4)	26.36 ± 3.39	38.08 ± 2.26	5.94 ± 0.17	4.70 ± 0.52	3.83 ± 0.47	0.42 ± 0.04	5.02 ± 0.17	2.32 ± 0.11	10^6–10^7	10^3–10^6

[a] Number of samples analyzed given in parentheses
[b] Total acidity expressed as percent lactic acid
[c] Values given indicate the range of counts obtained within each product type

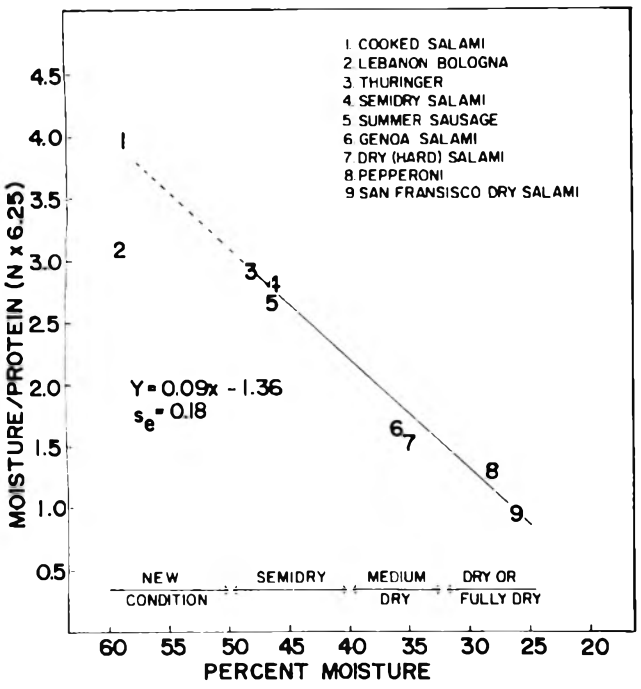

Fig. 1—Linear relationship between percent moisture and the moisture-to-protein ratio of some dry sausage products. (Cooked salami indicated is not a dry sausage product.) The standard error of estimate, S_e, represents one standard deviation from the predicted line values.

Standard deviations and linear regression analyses were those of Steel and Torrie (1960).

RESULTS & DISCUSSION

QUANTITIES of each chemical constituent shown in Table 1 are reflective of the end result of the degree of drying utilized in industry for each product. Although Lebanon bologna is frequently referred to as a semidry product (Kramlich et al., 1973; Komarik et al., 1974) there is no actual drying step in processing (Palumbo et al., 1973). However, steps involving aging and fermentation of Lebanon bologna and mellowing after extensive smoking are similar to practices used for some dry sausages.

The total acidities (calculated as lactic acid) given in Table 1 do not actually represent the true quantity of lactic acid. In using a titration procedure in several previous studies conducted at our laboratory (Keller et al., 1974; Keller and Acton, 1974; Acton and Keller, 1974), it was possible to correct for the total acidity occurring in the initial meat mix prior to fermentation. This "correction factor" is based on the inherent buffering capacity of muscle tissues (Honikel and Hamm, 1974). No correction was applied in the acidities of this report since the buffering capacity of the meat mixes was unknown.

Due to the variance in total plate counts and lactic acid bacteria found among similar products from different manufacturers, only the range of bacterial counts is listed in Table 1. It should be noted that there was a narrow range in counts between the same product samples of a company collected at different time intervals. This indicates considerable control within each processor's plant to meet individual quality standards. The broad range of counts should be considered in any proposal for determining microbiological standards for regulatory purposes.

Currently there are two classification methods used for dry sausages. Wilson (1960) used a range of weight loss or "shrinkage" values for sausage classification and assigned different dryness descriptions for each shrinkage stage. Kramlich (1971) used the approximate moisture content of sausages. Since sausage preparations will vary in initial moisture content due to the type and quantity of meat tissues and trimmings used, an alternate method may provide more flexibility by combining both the terminology of Wilson's (1960) method and the moisture values of Kramlich's (1971) method.

The proposed alternate method is quantitatively based on the change in the product's moisture-to-protein ratio as the moisture content of the product decreases. Qualitatively, the descriptive dryness terminology is included over the range of moisture values found to exist during drying. Figure 1 shows how the products analyzed in this study compare along a linear regression of the moisture-to-protein (total N x 6.25) ratio and percent moisture content. An R^2 value of 0.96 was obtained. Lebanon bologna, which is not subjected to drying, and cooked salami (not a dry sausage product) are plotted in Figure 1 only to indicate where potentially higher moisture-containing products locate relative to dried sausages. Data obtained for these two products were not included in the regression analysis. The descriptive terminology for dryness classification is frequently used in industry to refer to the many varieties of salami style products that are available.

Use of the classification procedure can be enlarged to include other qualitative and quantitative characteristics such as firmness of product, actual weight loss ranges, and acid concentrations or flavor descriptions that occur within a specific manufacturer's process.

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Ms received 12/6/75; accepted 1/12/76.

Technical contribution No. 1285 of the South Carolina Agricultural Experiment Station, Clemson University, Clemson, SC 29631.

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A Research Note

SENSORY EVALUATION OF PORK SAUSAGE CONTAINING TEXTURED VEGETABLE PROTEIN

ABSTRACT

The objective of this study was to evaluate the flavor, juiciness, and general acceptability of pork sausage containing four levels of textured vegetable protein (VP). The different formulas were evaluated, one at a time, under red lights and sensory evaluations of juiciness, flavor, and general acceptability were made on a 5-point hedonic scale (1, like very much to 5, dislike very much). Mean panel scores for flavor indicated the formula containing 10% VP was most liked, followed in order by the control, 20% and 30%. However, the control and 10% treatment were superior to the 20 and 30% treatment. Judgement of juiciness indicated a significant difference between the 10% and 20% treatment, but no significant difference could be detected when comparing either the control or 30% treatment to the 10% and 20% treatments. There was no significant difference among any of the treatments of general acceptability, but the 10% was most liked by the panelists followed by the control, 30% and 20%. Mean panel scores for flavor, juiciness and general acceptability revealed that none of the treatments were disliked. The 10% treatment was the most preferred of the four treatments when flavor, juiciness, and general acceptability were evaluated.

INTRODUCTION

THE USE OF SOY and vegetable proteins as meat extenders has greatly increased over the past 5 yr. Anderson and Lind (1974), Baldwin et al. (1975), Bowers and Engler (1975), Williams and Zabik (1975), Drake et al. (1975) and Cross et al. (1975) have studied the use of soy proteins incorporated into various meat products. However, to date, reports related to the use of soy or vegetable protein in pork sausage have been limited. The objective of this study was to evaluate the flavor, juiciness, and general acceptability of pork sausage containing four levels of textured vegetable protein (VP).

EXPERIMENTAL

THE MEAT PORTION of the sausage was prepared by grinding pork trim and fat (1°C) through a 4.8 mm plate. Separate samples of these two products were analyzed for fat content by the modified Babcock method (Salwin et al., 1955). The fat level was adjusted to 35% fat in the final product. The four formulas (Table 1) contained 0 (control), 10%, 20% and 30% rehydrated VP (SURPO 50, Ralston Purina Co.).

The VP was rehydrated at a ratio of three parts water to one part VP, weight basis. A combination of oleoresins of black pepper, sage and ginger on a dextrose carrier was added and the mixture blended. The meat was added and thoroughly mixed. This product was then ground through a 6.4 mm grinder plate.

The sausage was formed into 100-g patties with a patty press and stored (1°C, 24 hr) until cooked. Patties from each treatment were cooked in separate electric skillets connected to a voltage regulator set at 110V. Surface temperature of the skillets are adjusted to 117°C. The patties, two per skillet, were cooked 4 min on each side, cut into six wedge-shaped pieces, and each wedge placed into a 50-ml preheated beaker. A total of 32 different judges evaluated the four formulas. Four panels were conducted with 8 judges each. For each panel, the order of serving was randomized.

The different formulas were evaluated, one at a time, under red lights and sensory evaluations of juiciness, flavor, and general acceptability were made on a 5-point hedonic scale. Numerical values were assigned to each of the five catagories from 1, like very much, to 5, dislike very much.

Analyses of variance (single classification) were applied to the data. To locate significant ($P < 0.05$) differences among means, Duncan's new multiple range test (1955) was used.

RESULTS & DISCUSSION

THE MEAN PANEL SCORES for flavor indicated that the formula containing 10% VP was most liked (mean 2.1), followed in order by the control (mean 2.2), 20% (mean 2.8), and 30% (mean 2.8), (Fig. 1). However the control and 10%

Table 1–Pork sausage formulae expressed as percentages

Treatment	Pork	Hydrated vegetable protein	Salt	Flavoring
0	98.06	0	1.62	0.266
10	88.21	9.94	1.62	0.266
20	78.41	19.6	1.62	0.266
30	68.61	29.36	1.62	0.266

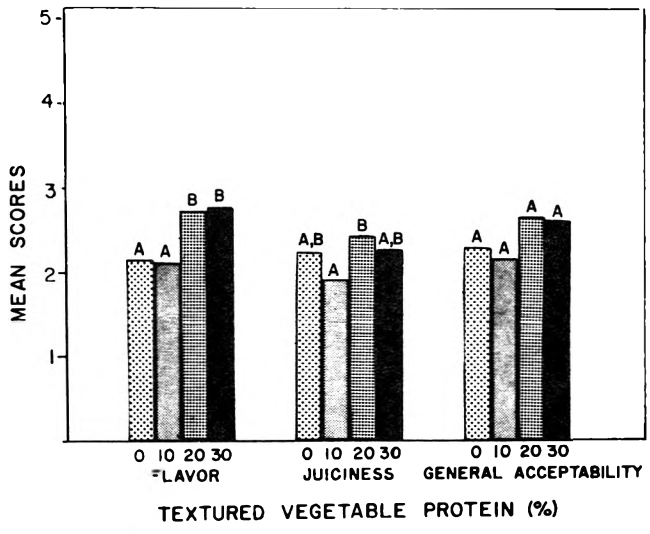

Fig. 1–Mean sensory scores (N = 32) for pork sausage containing textured vegetable protein. (Where letters within an attribute differ, means differ significantly, $P < 0.05$).

treatment were superior to the 20 and 30% treatment ($P < 0.05$).

These results were somewhat similar to the results of Baldwin et al. (1975) in which turkey patties and beef patties were extended with varying amounts of textured soy protein, similar to those used in this study. However in this research the 10% treatment was preferred in flavor over the control. Bowers and Engler (1975) reported that meaty flavor decreased with increased amounts of textured soy protein and Williams and Zabik (1975) reported a significant ($P < 0.01$) difference between 0 and 30% soy substituted pork loaves when flavor was evaluated. However when these levels of soy protein were formulated in beef or turkey loaves, there was no difference in flavor (Williams and Zabik, 1975).

The judgement of juiciness indicated that there was a significant ($P < 0.05$) difference between the 10% and 20% treatments. In contrast, no significant ($P < 0.05$) difference in juiciness could be detected when comparing either the control or 30% treatment to the 10% and 20% treatments.

It should be pointed out that the sensory method did not measure amounts of juiciness but only preferences for juiciness. Anderson and Lind (1975) found that the percent fat retained was more in all the nonextended beef patties. In addition, the percent moisture retained was larger in the extended patties. Drake et al. (1975) found textured soy protein did not have a binding effect on the fat present in the ground beef patties, but its addition resulted in a product with higher moisture, both before and after cooking. It can therefore be surmised that the preferences expressed for the control and 30% treatments were due to the percent fat retained in the control and percent moisture retained in the 30% treatment. The reasons for the 10% and 20% treatments being rated as they were must be related to the fat and water retention levels of the 10% and 20% formulae.

There was no significant ($P < 0.05$) difference of this general acceptability among any of the treatments. However, the general acceptability of the 10% treatment was most liked by the panelists, followed by the control (mean 2.3), 30% (mean 2.6), and 20% (mean 2.7) (Fig. 1). These results are similar to those of Cross et al. (1975).

Mean panel scores for flavor, juiciness, and general acceptability revealed that none of the treatments were disliked (Fig. 1). The 10% treatment was the most preferred of the four treatments when flavor, juiciness, and general acceptability were evaluated.

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Ms received 11/14/75; revised 1/16/76; accepted 1/20/76.

Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 7414.

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A Research Note

PROXIMATE ANALYSIS, PROTEIN QUALITY AND MICROBIAL COUNTS OF QUICK-SALTED, FRESHLY MADE AND STORED FISH CAKES

ABSTRACT

With the purpose of evaluating quick-salted fish cakes as a food, proximate chemical analyses, protein quality (Protein Efficiency Ratio and Net Protein Utilization) and bacterial counts of cakes, freshly made and stored for 18 months at an ambient tropical temperature, were determined. The cakes were found to contain an average of 30% protein, 60% salt and 10% moisture. Freshly made cakes were found to be superior, and 18-month-old cakes slightly inferior to casein, in both PER and NPU values. Finally, total plate and halophilic counts of freshly made cakes, already low (9,000 and 12,000/g, respectively), were found to decrease to almost zero after 18 months. Considering present U.S. Recommended Daily Allowances for high quality protein foods, it was calculated that each 450-g cake, costing $0.44, would be sufficient to cover the daily protein needs of three adults or possibly two adults and two young children. The average daily protein cost for each adult would be $0.15.

INTRODUCTION

PREVIOUS PAPERS have dealt with different aspects of a quick-salting process for fish (Del Valle and Nickerson, 1968; Del Valle and Gonzalez-Iñigo, 1968; Del Valle et al., 1973a, b). The product resulting from the process, quick-salted fish cakes, represents a low cost high protein food which has been suggested as a means for combatting protein malnutrition in developing countries. One advantage of the cakes is that, due to their low moisture and high salt contents, they require no refrigeration, even at tropical temperatures. The cakes are presently being manufactured and sold in Yucatán, Mexico, apparently with good acceptance by low income groups. Raw materials employed are low cost, normally nonutilized species. Present sales price is $0.44 per 450-g cake.

Although it has been believed that since quick-salted fish cakes are made from fish muscle they should possess a high nutritional value, no experimental determination of their protein quality has so far been made. Also, the microbial stability of cakes stored for long periods of time at ambient tropical temperatures has not been determined. [Del Valle et al. (1973b) determined bacterial counts of cakes stored for relatively short periods, 1–3 months.] This work, therefore, involved determinations of proximate chemical analysis, protein quality and bacterial counts of quick-salted fish cakes, both freshly made and stored for 18 months at an ambient tropical temperature.

EXPERIMENTAL

PROXIMATE chemical analysis involved determinations of moisture, protein and salt contents of the cakes as follows: moisture content by drying to a constant weight in a vacuum oven at 70°C; protein content by the Kjeldahl method (AOAC, 1965); and salt content by digesting in nitric acid, adding an excess of silver nitrate and back-titrating with ammonium thiocyanate, according to AOAC Method No. 18,008 (1965).

Two indices of protein quality were determined. Protein Efficiency Ratio (PER) and Net Protein Utilization (NPU) using casein as a reference protein (Munro and Allison, 1964; Miller, 1963). Five male weanling rats, 22–23 days old and weighing 27–28g were used in each determination. Before initiation of feeding, all animals were standardized by starving for 24 hr. All diets, including the casein diet, were standard formulations with protein level adjusted to 10%. The feeding period was 28 days in all cases. Rat weights and amount of food consumed by each rat were recorded daily during the duration of the test. All diets were analyzed by the Kjeldahl method (AOAC, 1965) in order to determine their protein contents. Body nitrogens for NPU determinations were measured by sacrificing the animals at the end of the test period, homogenizing carcasses and analyzing homogenates by the Kjeldahl method (AOAC, 1965). Body nitrogen at the beginning of the test period was taken equal to average body nitrogen of five of the same weanling rats (population, age and average weight) as those used in the feeding tests. Average PER and NPU values, with corresponding standard deviations, were finally calculated for each group of animals fed the same diet, based on individual values.

It should be added that cakes were desalted for inclusion in diets by leaching twice in boiling water. Salt contents of the leached cakes were determined according to AOAC Method No. 18,008 (1965).

Two types of bacterial counts were made as follows: (1) Total plate count, to obtain a measure of the general quality of the product, by plating dilutions from 10^{-1} to 10^{-4} in nutrient agar and incubating for 48 hr at 25°C. 25°C was selected as an incubation temperature because it was considered to be optimum for growth of contaminants of marine origin present in fish. (2) Halophilic count, to detect the presence of salt-tolerant microorganisms, by plating the same dilutions (10^{-1} to 10^{-4}) in nutrient agar containing 10% sodium chloride, and incubating at 37°C for 24 hr. The 10^{-1} dilution was prepared by mincing 10g of sample in 90 ml of sterile dilution water in a Waring Blendor.

Quick-salted fish cakes used in this work were prepared in the Yucatán plant described in a previous paper (Del Valle et al., 1973a) from "armado" (*Nicholsina usta*), a normally nonutilized species found in the Gulf of Mexico. This process used in the plant was as follows. After being taken out of the water, the fish were washed, eviscerated and filleted by hand. The resulting fillets were ground in a Hobart-Dayton Model 4612 meat grinder, equipped with a ½-HP motor and stainless steel parts, after which the ground material was weighed and mixed with an amount of salt equal to 70% of its weight. After allowing the ground salted muscle to stand for approximately 1 hr to allow for maximum water expulsion by salt, cakes were formed in the hydraulic press previously described (Del Valle et al., 1973a); 750g ground salted material was added to the press in order to form a wet cake weighing approximately 550g. The wet cakes were finally sundried for 3 days; each dry cake weighed approximately 450g. The dry cakes were packed as such in a cardboard box and stored for 18 months at room temperature, which varied from 18 to 42°C. Plant capacity was approximately 250 kg of dry cakes (550 cakes) per 8-hr day.

RESULTS & DISCUSSION

TABLE 1 reports results of proximate chemical analyses of the cakes. It may be seen that freshly-made cakes had a slightly higher moisture content than stored cakes, showing that the latter dried out during storage. Salt contents of both cakes

were fairly high and protein contents correspondingly low, reflecting the high salt dose used in making the cakes. It should be noted that high salt doses are employed because they have been found to produce drier and more compact cakes. On the other hand, dry cake salt content could be decreased and protein content correspondingly increased if desired, by using lower salt doses.

Table 1 also shows that, since moisture, protein and salt percentages added up to 100% in both cases, lipid contents of freshly-made and stored cakes were negligible. This was probably due to the fact that "armado" is a lean species; also, whatever oil was present in the muscle was probably pressed out when making cakes. It might be noted that cakes made from more fatty species would probably possess lower protein contents than those made from "armado" under similar conditions; on the other hand protein quality, which depends upon amino acid composition rather than upon lipid content would probably remain unchanged, since it is known that for fin species fish muscle aminograms vary little with species (Geiger and Borgstrom, 1962).

Finally, leached cake salt contents were found to average 2.0%, showing that the desalted products were completely edible.

Results of protein quality determinations are reported in Table 2. It may be seen that, as expected, both cakes had excellent protein quality. However, freshly made cakes were superior to stored cakes, and indeed were also superior to casein, while stored cakes were slightly inferior to casein in protein quality. Table 2 also shows that both indices—PER and NPU—behaved similarly. Statistical analysis of these results by application of t-tests revealed that all differences were significant at the 99% level.

The protein quality loss in stored cakes is not surprising, and was probably due to loss of lysine availability resulting from nonenzymatic Maillard-type browning reactions; stored cakes were somewhat darker in color than freshly made cakes.

Table 1—Proximate chemical analyses of quick-salted fish cakes, freshly made and stored at an ambient tropical temperature for 18 months, wet basis

Type of cake	Percent moisture	Percent protein	Percent salt
Freshly made	12.9	30.9	56.2
Stored	7.9	31.0	61.4

Table 2—Protein quality of quick-salted fish cakes, freshly made and stored at an ambient tropical temperature for 18 months

Type of cake	PER[a]	Corrected PER (Casein PER = 2.5)	NPU[a]
Freshly made	3.6 ± 0.4	2.7	89.9 ± 6.1
Stored	2.8 ± 0.4	2.1	77.9 ± 4.1
Casein	3.3 ± 0.5	2.5	82.3 ± 5.0

[a] PER and NPU values reported as mean ± standard deviation.

Table 3—Bacterial counts of quick-salted fish cakes, freshly made and stored at an ambient tropical temperature for 18 months

Type of cake	Total plate count per gram	Halophilic count per gram
Freshly made	9,000	12,000
Stored	—[a]	—[a]

[a] Count less than 30 on 10^{-1} dilution.

On the other hand, this loss was not too great, considering the long storage times involved. Also, PER and NPU values of stored cakes were very good, equal to or better than those of many processed soybean products presently available in the market, which are of the order of 2.0–2.1 (casein PER = 2.5) (Hamdy, 1974; Wolf and Cowan, 1971). Since storage times encountered in practice would normally not exceed 12 months, it may be concluded that quick-salted fish cakes would probably be a relatively high quality protein food.

Indeed, the effectiveness of quick-salted fish cakes as a human protein food may now be evaluated. For high quality proteins such as those of quick-salted fish cakes (PER equal to or greater than that of casein), the U.S. Recommended Daily Allowance has recently been set at 45g for adults (Briggs, 1974). Since dry cakes have been found to contain an average of 30% protein, it may be calculated that each dry cake weighing 450g and costing $0.44 contains 135g of protein. This amount would be sufficient to cover the daily needs of three adults, or possibly two adults and two young children, allowing an average of 25g protein per day for each child. The average daily protein cost per adult eating fish cakes would then be $0.15.

Table 3 reports results of bacterial count determinations. It may be seen that counts of freshly made cakes, already low, decreased appreciably during storage. These results confirm the fact that, from a microbial point of view, quick-salted fish cakes are stable without refrigeration, even when stored at tropical temperatures. It might be noted that fresh cake counts were similar to those obtained in previous work (Del Valle et al., 1973b).

CONCLUSIONS

IT HAS BEEN ESTABLISHED that quick-salted fish cakes are a good protein food, both in quality and in quantity. Qualitywise, freshly made cakes that have been desalted surpass casein, while cakes stored during 18 months at an ambient tropical temperature and then desalted are slightly inferior to casein in PER and NPU values. Quantitywise, presently produced cakes contain approximately 30% protein, 60% salt and 10% moisture. Under these conditions, each 450-g cake costing $0.44 is sufficient to satisfy the daily protein needs of three adults, or possibly two adults and two young children.

Finally, quick-salted fish cakes have been found to be safe for human consumption from a microbial point of view, as evidenced by the fact that total plate and halophilic counts, already low in freshly made cakes, drop to almost zero after storage for 18 months without refrigeration at an ambient tropical temperature.

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Ms received 9/18/75; revised 12/16/75; accepted 12/25/75.

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A Research Note

WATER BINDING OF COOKED FISH IN COMBINATION WITH VARIOUS PROTEINS

ABSTRACT

Water binding of cooked fish was determined in combination with (a) sodium, calcium, potassium and isoelectric form of soy protein isolate; (b) sodium and calcium whey protein concentrate; and (c) sodium caseinate. The sodium proteins were added at 0%, 2%, and 5% level, the other proteins at 0% and 2% level. Water binding was determined with a gravimetric adaptation of the filter paper press method. All proteins increased the water binding of cooked fish. The fresh and 2-yr old samples of sodium soy proteinate were significantly better water binders than the same proteinate aged for 8 yr. The ions associated with the soy and whey proteins did not affect the water binding significantly. Sodium soy proteinate and sodium caseinate were both better water binders than the sodium whey proteinate.

INTRODUCTION

ONE REASON WHY the leftover parts of fish carcass, after the fillets have been removed, are underutilized as a source of fish flesh is the poor water binding quality of such minced flesh. A product with a "weepy" texture will have little consumer appeal. Another short-coming is the relatively low flavor acceptance of fish products. In order to improve these qualities, fish has been combined, e.g., with beef (King and Flick, 1973), but also with other texturizing additives.

Consumer qualities of foods, such as appearance, flavor, as well as drip and shrinkage on cooking, depend greatly on the degree of water binding. Therefore, when the water binding of a product is improved, its consumer acceptance is generally increased.

Vegetable and milk proteins have been used in meat products as water binders for quite a long time. Their water binding qualities have been evaluated extensively (e.g., Thomas et al., 1973). Soy protein has gained recognition not only as a good protein source, but also as a good water binder, texturizer, stabilizer and adhesion agent. Spray-dried whey protein, a by-product of the cheese industry, is another source of high-quality protein and its water-binding properties have been reported in the literature (Berlin et al., 1973). The milk protein, casein, has a long history of use as a water binder in the food industry.

With growing demand for inexpensive, high-quality protein products, fish products should gain in importance provided that the texture and flavor are improved.

This study was performed to investigate the water-binding properties of various binder proteins in ground cooked fish to provide information for fish product development.

MATERIALS & METHODS

IN THIS STUDY, various commercially available soy protein isolates, whey protein concentrates, and caseinate were combined with ground fish. Soy proteinates, although at different age levels and ionic forms, were specially prepared research samples. The experimental design is given in Table 1. Several pounds of haddock fillets were ground and blended in a Waring Blendor at high speed. Weighed portions of fish and binder, without added water or salts, were blended in an electric micro-blender. An amount of 40g of the mixture was sealed in a 2-11/16 in. × 11/16 in. can. The sample cans were refrigerated for 24 hr, to allow the

Table 1—Fish and binder protein combinations used to determine water binding

Sample no.	Percent fish	Percent binder protein added	Type of binder proteins	Age of binder protein	% Solid content in sample
1	100	–	–	–	19.2
2	98	2	Promine-D, a sodium soy protein isolate (Central Soya)	fresh	21.2
3	98	2	Promine-D	2 yr	21.2
4	98	2	Promine-D	8 yr	21.2
5	98	2	Promine-C, a calcium soy protein isolate (Central Soya)	2 yr	21.2
6	98	2	Promine-K, a potassium soy protein isolate (Central Soya)	2 yr	21.2
7	98	2	Promine-R, an isoelectric soy protein isolate (Central Soya)	2 yr	21.2
8	95	5	Promine-D	fresh	24.1
9	98	2	Sodium Protolac, a whey protein concentrate (Borden)	fresh	21.2
10	95	5	Sodium Protolac	fresh	24.1
11	98	2	Calcium Protolac	fresh	21.2
12	98	2	Sodium caseinate (Deltown Chemurgic Corp.)	fresh	21.2
13	95	5	Sodium caseinate	fresh	24.1

Table 2—Effect of various binder proteins on water binding of cooked fish.

Sample group	Samples compared[a]	Juice released onto filter paper (gram per gram of sample)[b,c]				
I	1, 2, 3, 4	Control	2% Promine-D fresh		2% Promine-D 2 yr	2% Promine-D 8 yr
		0.472 ± 0.014	0.402 ± 0.015	=	0.403 ± 0.012	0.427 ± 0.010
II	1, 3, 5, 6, 7	Control	2% Promine-D		2% Promine-C	2% Promine-K 2% Promine-R
		0.472 ± 0.014	0.403 ± 0.012	=	0.413 ± 0.015	= 0.408 ± 0.013 = 0.409 ± 0.010
III	1, 9, 11	Control	2% Sodium whey protein		2% Calcium whey protein	
		0.472 ± 0.014	0.423 ± 0.011	=	0.415 ± 0.016	
IV	1, 2, 12, 9	Control	2% Promine-D		2% Sodium caseinate	2% Sodium whey protein
		0.472 ± 0.014	0.402 ± 0.015	=	0.387 ± 0.018	0.423 ± 0.011
V	1, 8, 13, 10	Control	5% Promine-D		5% Sodium caseinate	5% Sodium whey protein
		0.472 ± 0.014	0.311 ± 0.014	=	0.301 ± 0.012	0.354 ± 0.010

[a]Sample numbers taken from Table 1
[b]Mean ± standard deviation
[c]An equal sign between the sample means indicates that these means were not significantly different. Means between which there is no equal sign are significantly different at p = 0.05 level.

contents to equilibrate, and then heated for 35 min in a boiling water bath. The cans were stored at 4°C for 1 day before the determination of the water binding of the samples. Five samples were taken from each can. One sample was taken from the center and the remaining four from evenly spaced intervals along the circumference of the can.

The water binding was determined by the gravimetric adaptation (Karmas and Turk, 1975) of the filter paper press method (Wierbicki and Deatherage, 1958). In the gravimetric method, a 3/4 in. diam aluminum foil liner is placed between the sample and the filter paper to aid in the removal of the sample residue from the filter paper. After the sample (300 ± 10 mg) had been pressed on the filter paper between two plexiglass plates at 500 psi for 1 min, the sample residue was removed from the filter paper. The filter paper was then immediately weighed. The water binding was indicated by the amount of juice absorbed by the filter paper or released by the sample.

Mean values of released water were calculated for the five samples taken from each can. Within each group to be compared, a one-way analysis of variance was performed to determine whether there was any difference between the various sample means in the group. Significance was determined at p = 0.05 level. If the analysis of variance gave a significant F-value, Scheffe's test was applied in order to determine which cans were causing the high F-value. Significance was again determined at the p = 0.05 level.

RESULTS & DISCUSSION

RESULTS were expressed in grams of water adsorbed by the filter paper per gram of sample. The sample means with their standard deviations are shown in Table 2. The samples were grouped to compare the effect of age, concentration and differences in ions associated with the protein. The different proteins themselves were also compared. Means which were significantly different are indicated in the table. All samples to which a binder had been added showed significantly better water binding than the control sample which contained only fish. The control sample gave the highest water release, 0.472 ± 0.014 gram of juice released per gram of sample. Although the control samples had a lower solid content as is evident from Table 1, all the binder-containing samples still had a significantly increased water retention over the control if the data were calculated on their dry weight basis.

Group I of the samples in Table 2 compared the effect of age on the water-binding capacity of Promine-D. While the samples containing fresh and 2-yr old Promine-D did not give means which were significantly different, the samples containing Promine-D stored for 8 yr at room temperature had a significantly lower water binding than the fresher Promine-D. Although Promine-D is a dry product, containing only about 5% water, one may speculate that this water may have been involved in reactions resulting in crosslinking of protein chains. Crosslinking decreases the ability of the protein to interact with and hold water and, in turn, decreases its water-binding capacity. Thus excessive aging decreases the water-binding capacity of proteins.

Sample group II compared the water binding of sodium, calcium, potassium, and isoelectric soy protein isolate added to the fish at the 2% level. An analysis of variance revealed no significant differences between these different ionic forms of soy protein concentrate. A similar result was obtained in the sample group III. When the water binding of sodium whey protein concentrate and calcium whey protein concentrate added to the fish at the 2% level were compared, an insignificant F-value resulted. The type of ion associated with the protein had no detectable effect on the water binding probably because the ratio of ions in the protein binders added to the fish was too small to allow the ions to play any significant role in water binding.

In the last two sample groupings IV and V, Promine-D, soy caseinate, and sodium whey protein concentrate combined with the fish at the 2% and 5% level were compared. At the 5% level the differences in water binding between these protein binders became more distinct than at the 2% level of addition. Whey protein concentrate clearly had the lowest water-binding capacity. This may be due to the fact that while the soy proteinates and caseinate both had a protein content of approximately 90%, the whey protein concentrate had only about 60% protein (lactalbumin), the balance of the composition being mainly lactose, a disugar. Promine-D and sodium caseinate both were excellent water binders; no statistically significant difference could be detected between the two binders.

These results have a resemblance to the results obtained by Hermansson (1971). She investigated the swelling properties of these proteins. Though swelling and water binding are not identical phenomena, they both involve interaction with water. Hermansson similarly found whey protein concentrate to have the poorest swelling properties; however, she found soy protein isolate to have better swelling properties than sodium caseinate.

The present study indicated that sodium caseinate was a slightly better water binder than sodium soy protein isolate and that the difference increased at lower concentrations. This is in agreement with the results determined earlier with differential scanning calorimetry (Karmas and Chen, 1974); however, sodium soy protein isolate (Promine-D) became a significantly better water binder than sodium caseinate above 5% addition levels.

In the sample groups IV and V in Table 2, three further

comparisons can be made as to the effect of increasing concentration of the three sodium protein binders from 2% to 5%. In all three cases the increase of protein concentration produced a significant increase in the water retention capacity. If the data are recalculated on the solid content basis, the 5% addition produced a significantly higher water retention than the 2% addition of the same binder for Promine-D and sodium caseinate but not for sodium whey protein. This latter effect may be due to the lower protein content of the whey concentrate. Furthermore, one may speculate that the total effect of water retention by the cooked fish protein and binder protein systems was more pronounced at the 5% addition level by a synergistic interaction between the proteins. In addition to chemical water binding forces, relatively more water was entrapped by a stronger denatured protein matrix.

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Ms received 6/16/75; revised 2/6/76; accepted 2/8/76.

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